Cuttlefish (*Sepia officinalis*) camouflage in varying environmental conditions

Kerry Perkins

Submitted for the degree of Doctor of Philosophy
University of Sussex
September 2016

University of Sussex

Kerry Perkins

Submitted for the degree of Doctor of Philosophy

Cuttlefish (*Sepia officinalis*) camouflage in varying environmental conditions

Summary

Cephalopods first appeared around 500 million years ago. Since then they have developed from the external shelled ammonites, belemnites and nautiloid's to the soft bodied organisms we find today. By becoming soft bodied, protection which would have been provided by the shell was lost and a different approach to predator avoidance was adopted.

Modern day cephalopods such as octopus, squid and cuttlefish frequently use camouflage techniques to avoid detection. In addition to countershading, which is an often used camouflage technique by aquatic species, the presence of chromatophores allow a versatile and rapid response in relation to stimuli. Cuttlefish expression of these chromatic responses can be categorised into chromatic components. It is the intensity and combination of the expression which makes them an interesting organism to study, when looking at how the environment influences camouflage decisions.

In this thesis, I present six experiments looking at how external environmental factors influence camouflage in *Sepia officinalis*.

The first experimental chapter looks at how 3D objects and proximity play a role in not only camouflage, but behavioural responses. The first set of experiments discuss how factors such as contrast and size of an object may elicit different responses both behaviourally and chromatically. The proximity of the cuttlefish to the object was then

investigated to determine if visual input was a possible cause for the differences. Size of the object, proximity and contrast produced a differing response to that of a cuttlefish on a uniform background.

The two subsequent chapters look at differing light information and whether cuttlefish treat these differences similar to that of low contrast. Reaction to turbid and low light levels show similar responses in camouflage, suggesting that similar mechanisms are employed when there is reduced light and high scatter information. In respect to luminance versus reflectance, cuttlefish seem to be able to differentiate between a projected and reflected image where they appear to treat projected images like a lower contrast value.

The last experimental chapter investigates motion camouflage in respect to predation. Prey and distance had a large effect on behaviour and how camouflage was expressed. Over greater distances behavioural variance reduced. Darkening of the head region and arm waving was also present over a greater distance. Camouflage varied in relation to background with a more uniform background producing reduced expression when moving. Stationary predation therefore elicits a different response than that of motion camouflage in cuttlefish.

Declaration

I hereby declare this thesis has not previously been submitted, either in the same or a different form to this or any other university for a degree.

All of the experiments, analysis, and interpretation described within this thesis are my own.

Acknowledgements

None of this could have been achieved without the love and support from my parents. Their unrelenting support and interest in a subject completely alien to them has meant so much to me and I am forever indebted to them. The support and resources that have been afforded to me by Sea Life Brighton has made this journey an eventful and successful one and I would especially like to thank Max for letting me run with a crazy idea and building up research in the centre.

The support, guidance and overall opportunities Daniel has given me through this process has allowed me to grow professionally and as a scientist. I wish to thank him for helping me through the many years it has taken to get to this point.

Lastly, I would like to thank Josh who has shown me support and love can drive you to reach the highest of aims and dreams, without you this journey would have been so much harder.

Contents

Chapter 1: General Introduction

1.1 Environment conditions

1.1.1 Aquatic environment

Water makes up 70% of the worlds surface with only 1% of this being freshwater, making the world oceans one of the most varied and abundant habitats. The sea floor can be divided into the continental shelf, continental slope and the abyssal plain. All three of these environments provide different challenges and habitats to the organisms that live in them. The continental shelf only accounts for 3% of the global ocean area, but even so benthic marine habitats extend from the shore to the shelf break, which is on average 130km from the coast (Johnsen 2012). This environment is highly variable with wide temperature fluctuations, high sediment settling and relatively high current and tidal oscillations.

1.1.2 Optical properties of sea water

Optical properties of natural seawater are quite different to those of air and even of pure water, due to the presence of particles and dissolved absorbing substances (Johnsen 2012). Light is both scattered and absorbed in water, with absorption having a strong wavelength dependence. The red and oranges with the shortest (UV) wavelengths are lost first, with blue and green penetrating to the greatest depths (Stramski *et al* 1991). The importance of this can be observed in relation to aquatic animal colouration. Many species that occur at deep depths are black or red where only the blue/green wavelengths reach. It is suggested this colouration is to modulate the animal's reflectance in the blue green spectrum. For instance, if you shine a blue green light the organism background matches well compare to that under red light (Marshall *et al 2003*) At a shallower depth this sort of colouration would be less useful in an environment with higher light intensity and a wider illumination spectrum.

In cases of animals that live in the shallower parts of the water column certain structures may be used by organisms to alter light. Strategies such as being a transparent organism mean that light is scattered and therefore provides some protection from predation. In other animal's, structures may be used to reflect light such as silvery appearance in fish, tapeta in eyes and the iridophores in cephalopods (Johnsen 2012)

1.1.3 Location of sepia

Cuttlefish is in the genus *Sepia,* which includes over 100 species. They are predominantly demersal in habitat and found around the globe, but are absent from polar regions and the North West Atlantic. *Sepia officinalis,* the subject of this Thesis, are widespread in geographical distribution (Compagno 2001). In the English Channel adults migrate from deep water where they spend the Winter to localised spawning grounds along the coast of France and England during April. Males arrive before females, and large scale hatching occurs in August. Afterwards *S. officinalis* move off inshore waters into central areas of the channel. Life span can be anywhere from 12-24 months which seems to suggest that there are long and short generation cycles dependent on time of maturity.

1.2 Cephalopod body form

1.2.1 Basic Biology

The coleoid cephalopods - squid, octopuses and cuttlefish - share many features in their sensory apparatus including: mechanoreceptors, 'lateral lines', touch and pressure receptors, chemoreceptors and photoreceptors (Hanlon and Messenger 1988). These sensory inputs give a rich source of information about the environment. In addition to the usual response animals use, coleoids

can control remarkable changes in visual appearance, especially skin coloration patterns, and surface texture. They use these patterns for camouflage and communication, offering an insight into how these remarkable animals sense and especially see their environments. These behaviours are the subject of this Thesis.

1.2.2 Cuttlefish skin

Unlike the Nautilus that have retained their external shell, cuttlefish have internalised their 'shell' in the form of a cuttlebone. Due to the soft body nature, cuttlefish - like other coleoids - have developed other mechanisms to deal with predatory threat. One of these mechanisms is to use chromatophores, iridophores and leucophores located in the epidermis for camouflage (Figure 1.1). Chromatophores consist of a granulated elastic pigmented sac which is expanded by radial muscles, and otherwise collapses to a point. This means motoneurons running directly from the brain allow a mixture of colour to be displayed on the epidermis of the cuttlefish, to vary its intensity and patterns. Beneath the chromatophores are matte white leucophores and mirror-like iridophores which produce a white background for the chromatophores (Hanlon and Messenger 1988). Combining these different types of cells allows the cuttlefish to change their appearance, to first to prevent detection by predators, and then to deter them with warning signals (Langridge *et al* 2007; Langridge 2009).

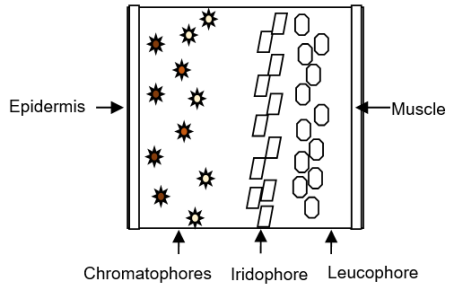


Figure 1.1 Skin arrangement of elements that contribute to body patterning in cephalopods

1.2.3 Cuttlefish vision and the eye

The eye in cephalopods is considered an excellent example of convergent evolution with that of underwater vertebrates (Land and Nilsson 2002). However, unlike the vertebrate eye which has a large amount of processing occurring in the retina, cuttlefish eyes process visual information outside the eye in the optic lobe. The eye also differs due to the presence of just one visual pigment effectively making the cuttlefish colour blind (Ferguson and Messenger 1991). This combination of camouflage ability and vision makes cuttlefish an interesting organism to study.

1.2.4 Camouflage in the aquatic environment

Stevens and Merilaita (2011) describe crypsis as ‘all traits that reduce an animal’s risk of becoming detected when it is potentially perceivable to an observer’. Camouflage relies on making oneself inconspicuous in the environment through strategies such as background matching and disruptive patterning (Stevens 2007). A habitat or lifestyle that includes high variation of the visual environment may benefit animals that can vary their camouflage. An example of this would be in animals such as chameleons, flounders and cuttlefish (Stevens and Merilaita 2011). This necessity to change in relation to one’s environment may be dependent on the environment itself being variable.

It is striking that the animals who are best at varying their appearance for camouflage are flatfishes and benthic cephalopods. Ocean environments can be highly variable, so large amounts of visual information are needed when making camouflage decisions. Organisms living in benthic environments may take the substrate into consideration when concealing themselves from potential predators and prey. Background matching, obliterate shading and disruptive

colouration have all been suggested as camouflage techniques (Stevens 2007), which would help conceal the organism against the sea floor. The ocean however is not a 2-Dimensional environment. Both horizontal and vertical information is present in the visual fields such as rocks and seaweed. How much an animal would take 3-Dimensional structures into consideration for camouflage may depend heavily on their visual acuity and their ability to camouflage. In relation to 3-Dimensional structures a masquerade response, where the animal resembles a commonplace object rather than the background, may be more suitable for an aquatic organism. *Monoclirhus polyacanthus* an Amazonian fish resembles leaves of the Amazon forest. Skelhorn *et al* (2011) showed that even in the absence of crypsis, masquerade had additional benefit in brimstone moth caterpillars. They also suggested that cognitive strategies of the predator, rather than sensory capabilities, are the selective force driving evolution of masquerade. This would suggest that if an animal is searching for their prey in an aquatic environment, their recognition and identification of the prey would be more important than the visual abilities of the animal. Distance of the predator to the prey may also influence detection. Colour patterns of some animals may therefore be distance dependent, conspicuous in close proximity and camouflaged from a distance (Stevens *et al* 2011)

Being cryptic can be effective when stationary due to strategies such as background matching. This strategy however relies heavily on the animal being similar to the background they are situated in (Stevens *et al* 2011). Once the animal moves the concealment that may have existed is often lost. In turn this increases the likelihood of an attack by a predator. Studies have suggested that some animals have developed certain camouflage strategies to confuse predators. Markings such as contrast bands, stripes and zig zags may reduce the predator's judgement of speed and trajectory (Stevens *et al* 2011). These strategies may however come at a cost due to the markings being conspicuous when stationary. In an animal which can change

their camouflage the strategy of banded markings may not be as necessary. When stationary the animal could use a more concealing camouflage technique and a different motion based camouflage technique when moving.

1.2.5 Aims of this thesis

Chapter 3: Camouflage in complex visual environments

As discussed earlier in this chapter the aquatic environment is highly variable. It is this along with the cuttlefish's ability of rapid camouflage that we wish to investigate in this thesis. Firstly, we will test a more natural situation with rocks of varying contrast and sizing. This will test behavioural and camouflage against a natural 3-Dimensional object. As the cuttlefish will be able to move within an arena with the rocks a further study looking at proximity of information will test whether the proximity of an object has influence on camouflage. It is hypothesised that there will be an influence of the objects and their distance on the cuttlefish camouflage expression.

The aims of Chapter 3 are:

1.To record the behavioural response to the presence of varying stone contrast and sizes

2.Determine the effect of a 3-Dimensional stone characteristics of contrast and size on camouflage

3.To examine how distance of varying checkerboard backgrounds on a vertical and horizontal axis influence expression of camouflage

Chapter 4: Effects of viewing conditions on camouflage

Camouflage is highly reliant on visual information and therefore it is important to understand how reduction of this information may affect camouflage. In situations such as low light and high turbidity visual information is reduced to the eye due to the reduction of light or high scatter in the latter. The influence of these on camouflage has only been tested in a couple of experiments Allen *et al* 2010, Cartron *et al* 2013 and has yet to determine if the reduction of information results in similar camouflage of a low contrast environment. A comparison between low contrast, high turbidity and low light is tested and discussed in Chapter 4. It is hypothesised that turbidity, low light and low contrast may influence camouflage expression in similar ways.

The aims of Chapter 4 are:

1. To determine the role turbidity has on cuttlefish camouflage

2. To investigate how low light effects camouflage

3. To compare any similarities of camouflage between lowlight and turbidity and that of low contrast

Chapter 5: Shadow Perception

After investigating low light and high turbidity which is reduced information to the eye in the aquatic environment, a further investigation into how light information is transmitted to the eye would further our understanding on the camouflage instigated. Reflectance versus luminance was tested by casting shadows. Shadows would naturally be cast into an aquatic environment, which would create natural luminance compared to bouncing off an object (reflectance).

However, little is known how these effect camouflage decisions. In Chapter 5 shadow perception is investigated in a preliminary study to test whether shadows are observed as low contrast, or are treated with a different form of camouflage. It is hypothesised that cuttlefish will treat the two treatments differently.

The aims of Chapter 5 are:

1. Can cuttlefish differentiate between low contrast, projected image and reflectance

2. How do cuttlefish respond to a projected image

Chapter 6: Motion Camouflage

Whereas the other experimental chapters have dealt with stationary camouflage the effect of motion on expression of camouflage is investigated in this chapter. There has been an increased interest in camouflage with motion in cuttlefish over the last few years Zylinski *et al* 2009b, Staudinger *et al* 2013. However only a few have looked at feeding in relation to behaviour and camouflage combined (Adamo *et al* 2006, Messenger 1968). Do cuttlefish use motion camouflage during prey capture and does it differ dependent on species and background? It is hypothesised that the behaviour and camouflage expression will change over distance, different backgrounds and prey items.

The aims of Chapter 6 are:

1. To determine the behaviour of prey capture over distance and substrate

2. To describe and analyse the different camouflage expression during predation

3. To investigate how prey items effect camouflage

Chapter 7: General Discussion

Finally, the overall trends and results will be compared and related to environmental conditions cuttlefish may encounter. In addition, the context of which these experiments relate to the larger picture of cephalopod camouflage and other animals will be discussed.

Chapter 2: Cuttlefish Husbandry and Experimental Methodology

2.1 New European Union legislation

On the 9th of November 2010 the European Directive on the protection of animals used for scientific purposes (Directive 2010/63/EU) came into force. With the implementation of the directive came a new set of regulations of which for the first time included cephalopods into a regulatory framework. The set of new regulations were transposed into national legislation and came into effect on the 1st of January 2013.

Vertebrates have been included under regulatory frameworks in research to promote excellent animal welfare for many years. However, apart from the United Kingdom which had included Octopus vulgaris as an 'honorary' vertebrate, cephalopods were not included until now. With the new inclusion to a legislative framework, welfare and guiding principles and humane end points need to be discussed and implemented within the cephalopod research community (Home Office 2014).

All the experiments in this thesis were either conducted before the implementation of the directive or did not require a licence to be conducted since implementation.

2.2 Husbandry of cuttlefish

The constraints and methods of keeping cuttlefish seem to differ dependent on the experimentation needs of the individual research laboratory. However, there are some basic and necessary standards which would lead to good overall husbandry practice and therefore better cuttlefish welfare.

2.2.1 Water parameters

Water quality is vitally important when keeping cuttlefish in any lab situation. Like any aquatic animal being housed, biological waste should be monitored and controlled. Ammonia (NH3) being the most toxic of the waste products should be maintained at undetectable levels of <0.5mgL this can be done by either filtration of the water, water changes or a constant turnover of water. Nitrite (NO2) and Nitrate (NO3) also should be maintained at low levels of <0.2mgL with the latter being less toxic and being able to be maintained up to 80mgL. There are varying thoughts on the limits in how european cuttlefish can be maintained (Figure 2.1)

	a)Guidelines -Sepia sp	b)Biaza guidelines	c)*S.officinalis* Cephalopod Culture
Temperature	*12-25*	*10-23*	*13-24*
Salinity	*29-33*	*30-35*	*33-37*
pH	8.2	*no data*	*no data*
Max NH3	*<0.5mgL*	*0*	*no data*
MaxNO2	*<0.2mgL*	*<0.3*	*no data*
Max NO3	*<80.00mgL*	*<25.00mgL*	*no data*
Min O2	*7.0ppm*	*no data*	*no data*

Figure 2.1: Review of recent husbandry literature in relation to parameters a) Fiorito *et al* (2014) b) Slater *et al* (2013) c) Iglesias *et al* (2014)

2.2.2 Housing

The amount of space required for keeping cuttlefish is often debated (Figure 2.2). Dependent on the needs and resources of laboratory, stocking numbers can vary greatly. For example, a lab requiring a few studies but not wishing to conduct aquaculture may only keep a few individuals. This may make it easy for individual identification and take up only a small amount of space due to individual housing of the cuttlefish. However, if several cuttlefish are required for study where individuals are not kept separately, this can cause difficulty due to identification, infighting, damage due to over stocking and disease.

In cases where a round vat is available this may be the best option if there is a lot of space, however this is more likely to be an option in an aquaculture facility rather than a research space. Glass tanks or rectangular holding tanks are probably more available. Both have merit although depending on size, problems can be caused as the cuttlefish get bigger due to damage to cuttlebone or mantle epidermis through repeated swimming into the side (Hanlon and Messenger 1996). Glass tanks may also not be suitable due to habituation of the cuttlefish to humans and their surroundings, dependent on the study.

Species	a) Guidelines	b) Home office 2014	c)Biaza Work Group	d) *S. officinalis* Cephalopod culture
Cuttlefish	Grouped	Grouped	Grouped	Grouped
Ratio- Juveniles	200 per m^2	No guidance	10 per sq foot	120 m^2 with minimum area of 1083cm^2
Ratio- Adults	2 per m^2 - adult	No guidance	20 x1000L- 2months 10 x 1000L 3 months 4x 1000L 6 months	30DAH to 10g

Figure 2.2: Ratio of cuttlefish grouping a) Fiorito *et al* (2014) b) Home office (2014) c) Slater *et al* (2013) d) Iglesias *et al* (2014)

2.2.3 Substrate

Substrate can also be a contentious issue dependent on the study. Studies requiring naivety may reduce substrate or backgrounds to eliminate bias. Whether this is depriving the cuttlefish of their natural behaviour of burying into substrate is unknown. Using sand at the bottom of tanks however can prove to be problematic, sand provides a filter for the waste of the cuttlefish, however it is also a source of possible contamination. There has however, been suggestions that using images of substrates or hides could provide some relief (Cooke and Tonkins 2015). In either case if not maintained properly it can produce less than optimal conditions which may lead to disease or stress of the animal.

2.2.4 Lighting

Lighting seems to be less important but the maintenance of a day night cycle should be implemented. Low lighting may be better than bright overhead lights and cuttlefish are less startled in these conditions.

2.3 Growth and Ontogeny

2.3.1 Nutrition

Food can greatly effect survival rate and growth of the cuttlefish. Dead food in early stages of development often produce high mortality rate and poor growth with individuals being considerable smaller (Navarro *et al* 2014). However, a mixture of live and dead food produces similar results to solely live food. This is beneficial if live food becomes scarce or one wants to reduce the cost of purchasing live food. There have been trials with artificial diet, but, as of yet have none have been brought to market (Navarro *et al* 2014)

2.3.2 Growth

Growth is dependent on amount or nutrition of the available food. If live food is fed continuously then growth can be considerable. A mixture of dead and live food may reduce the growth rate due to the decrease in the nutritional value, which is caused by freezing or storing dead food.

2.3.3 Lifespan

Cuttlefish lifespan differs considerably dependent on environmental factors and food constraints. Water temperature can increase cuttlefish activity and therefore food consumption, resulting in a faster growth rate and in some cases reaching of sexual maturity faster. In Portugal individuals often become sexually mature at 8 months (Sykes per comm) whereas in England it can be anywhere from 12-18months before reproduction occurs despite the opposite sex being present.

2.3.4 Damage and disease

Being a soft-bodied organism cuttlefish are more susceptible to damage and possible infection. Poor housing conditions where cuttlefish can damage themselves can lead to broken or damaged cuttlebones. Broken skin can also be present which can allow bacteria or infection to colonise the affected area. If not caught early infection can kill cuttlefish exceedingly fast (Sykes and Gestal 2014). The type of treatment is highly debatable due to no specialised drugs being available and it is often a trial and error due to the properties of the antibiotic Oestmann *et al* (1997)

2.4 Drug Case Study

2.4.1 Background

Cuttlefish are soft bodied and as such are at a higher risk of getting damaged or colonisation of bacteria around wounds. Until recently most of the disease and infections that had been identified and suitable treatments were listed in the DOMA guidelines (Hochberg and Kinne 1990). However, as our knowledge of infection and cuttlefish immune response increases new trials of drug treatments are being implemented.

2.4.2 Presenting signs

In this case three Cuttlefish presented with damage to the epidermis on the mantle and floating at the top of the water column. The presenting signs suggest it was some sort of bacterial infection.

2.4.3 Treatment

In consultation with International Zoological Veterinary Group (IZVG) it was decided to try a different treatment of Baycox 300mg solution in water at a concentration of 100mg per 100 Litres. A 50% water change was conducted each day.

2.4.4 Results

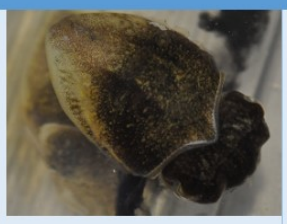
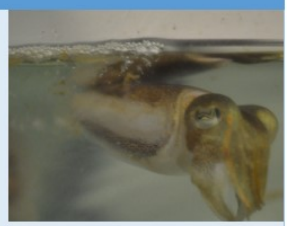
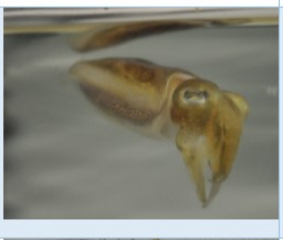
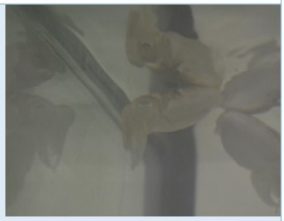
Time			
Day 1- Identification of problem		Day 5	
Day 2 -Start of treatment		Day 6	
Day 3		Day 7	
Day 4		Day 8- Last day of treatment	
Day 21- 3 weeks after initial signs			

Figure 2.3*:* Treatment with Baycox over 7 days and results after 3 weeks from initial presentation of signs

2.4.5 Discussion

Out of the three cuttlefish that were treated, two were treated successfully and lasted through to maturity after treatment without any additional treatments needed. The deceased cuttlefish was sent for pathology to identify the cause of the symptoms. The mantle lesions contained bacteria consistent with vibrio's along with what resembled Ichthyobodolike bodonid organism. After initial treatment, there appeared to be epidermis damage and subsequently abnormal chromatophore function. As the cuttlefish developed however the chromatophore function returned and the epidermis repaired. Behaviour of the cuttlefish did not seem to be effected after the initial treatment and there was little differentiation between those treated and other cuttlefish of similar age.

2.5 Set up of Experimental tank

Cuttlefish were housed in a purpose-built tank at Brighton Sea Life centre, UK described by Kelman (2007). The holding tank was constructed of grey 9mm PVC of 2000mm x 900mm x 600mm dimension. Within the holding tank the internal space can be divided into 6 sections when needed, with a flow through ability to a stand pipe in the farthest left section. Each section contains an airline and stone. There are also three valves of saltwater feeds inputting water into the tank from an open system of coastal saltwater. Overhead fluorescent room lights were used on a 10L:14D and tanks were syphoned daily.

Filming was conducted in a designated filming tank of 900mm x 750mm x 150mm (Figure 2.4). A black aluminium hood surrounded the top of the filming tank with a mirror set above the tank at 45 angle to reduce disturbance to the cuttlefish during filming. Lights were two 30w fluorescent bulbs down either side of the tank and five 20w

halogen bulbs. There was a viewing window which was used to taken video and pictures. Video was taken with a Sony video camera HDR-XR100 and still photos with a Nikon D500.

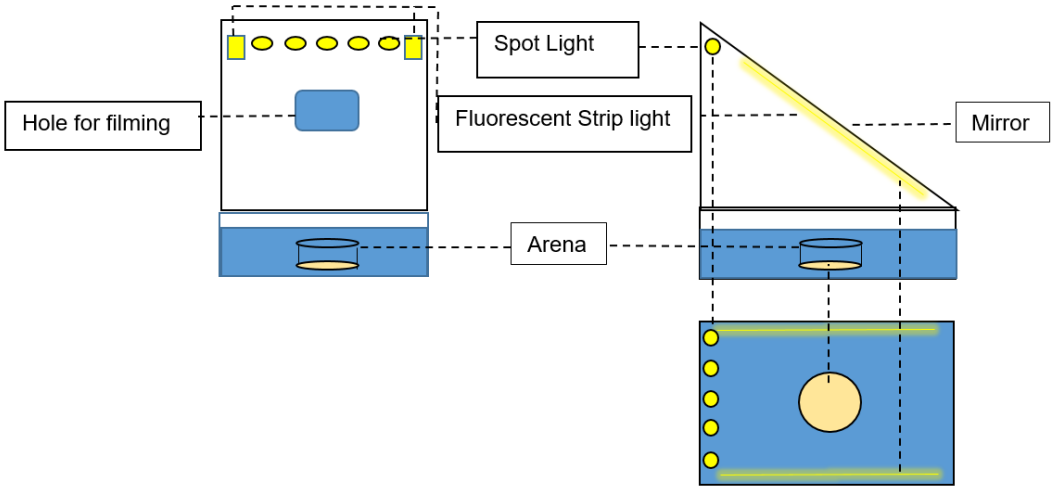
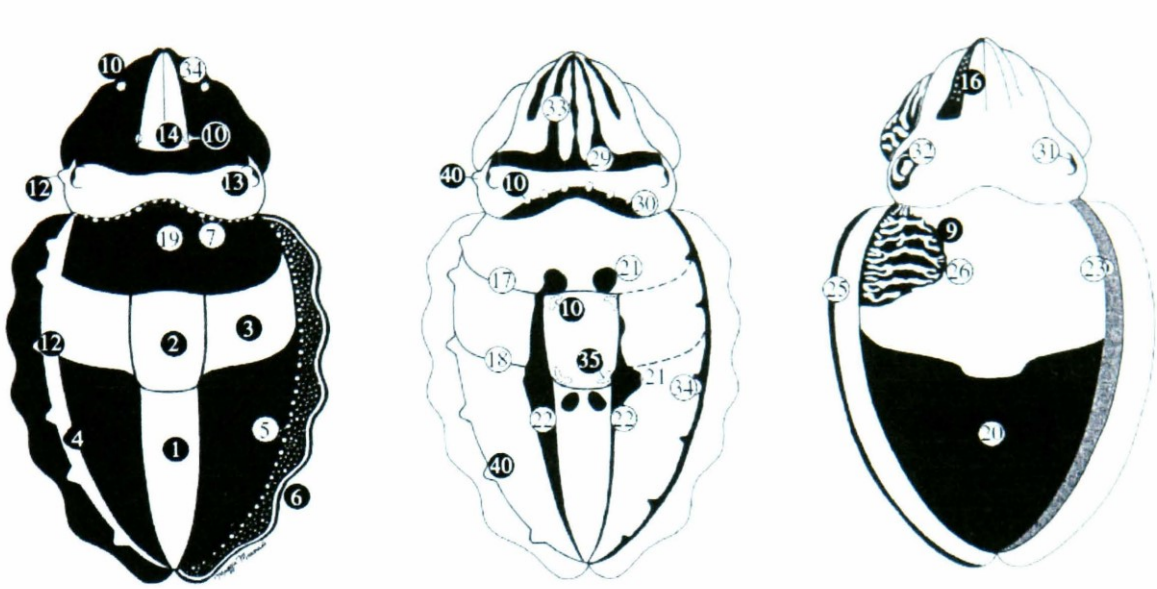


Figure 2.4: Set up design of filming tank

2.6 Grading of images

Components were used from an existing list like that used by Zylinski (2009) and described in Hanlon and Messenger (1988) (Figure 2.5). Cuttlefish images were graded by eye on a three-point scale with scores being entered into a SPSS v22 spreadsheet to be analysed. When a component was expressed a score was noted, with the most heavily expressed component having the highest score, in comparison absence of a component was scored as 0.



CHROMATIC COMPONENTS

Light

(1) White posterior triangle
(2) White Square
(3) White posterior triangle
(4) White lateral stripe
(5) White fin spots
(6) White fine line
(7) White neck spots
(8) Iridescent ventral mantle
(9) White Zebra bands
(10) White landmark spots
(11) White splotches
(12) White major lateral papillae
(13) White head bar
(14) White arm triangle
(15) Pink iridophores arm stripes
(16) White arm spots (males only)

Mantle

Head or Arms

Dark

(17) Anterior transverse mantle line
(18) Posterior transverse mantle line
(19) Anterior mantle bar
(20) Posterior mantle bar
(21) Paired mantle spots
(22) Median mantle stripe
(23) Mantle margin stripe
(24) Mantle margin scalloping
(25) Dark fin line
(26) Black zebra bands
(27) Mottle
(28) Latero-ventral patches
(29) Anterior head bar
(30) Posterior head bar
(31) Pupil
(32) Eye ring
(33) Dark arm stripes
(34) Dark arms

Texture Components

(35) Smooth skin
(36) Coarse skin
(37) Papillate skin
(38) Wrinkled first arms
(39) White square papillae
(40) Major lateral papillae

Postural Components

(41) Raised arms
(42) Waving arms
(43) Splayed arms
(44) Drooping arms
(45) Extended fourth arm (males only)
(46) Raised head
(47) Flattened body
(48) Flanged fin

Locomotor Components

(49) Sitting
(50) Bottom suction
(51) Buried
(52) Hovering
(53) Jetting
(54) Inking

BODY PATTERNS

Chronic

(1) Uniform light
(2) Stipple
(3) Light Mottle
(4) Dark Mottle
(5) Disruptive (weak, strong)
(6) Weak Zebra

Acute

(7) Uniform Blanching
(8) Uniform Darkening
(9) Acute Disruptive
(10) Flamboyant
(11) Dermatic
(12) Intense Zebra (male only
(13) Passing cloud

Figure 2.5: Components described by Hanlon and Messenger 1988. Notice the large array chromatic components. Taken from Hanlon and Messenger (1988)

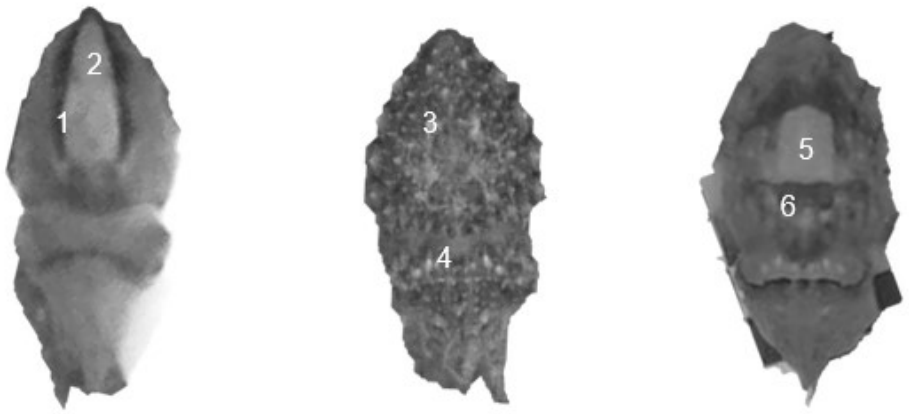


Figure 2.6: Examples of components identification. 1) Median mantle stripe 2) White mantle triangle 3) White landmark spots (white square) 4) White landmark spots (head) 5) White Square 6) Paired mantle spots anterior

2.7 Statistics

A Principal Component Analysis (PCA) was conducted on the grading scores and subsequent component regressions analysed by ANOVA. Post Hoc Bonferroni tests were conducted to determine more detailed significance. Principal Component Analysis is excellent for large multivariate data sets, which due to their size can prove difficult to recognise trends and often complex in relation to the data (Figure 2.7). The dimensions of the data are reduced so that similar trends can be recognised and grouped together. The list of similar components is produced by the degree of covariance between sets of the original variables. This has been proven to be an excellent method for investigating cuttlefish camouflage due to the array or expression and combinations possible. (Kelman 2007). The components are derived by eigenvalues. A larger eigenvalue suggests a larger share of the combination of characteristics. The largest eigenvalue will be termed Principal Component 1 and will have the largest of the eigenvalues. Once the eigenvalue drops below 1 the relatedness and the size of degree of the covariance also drops (Figure 2.8). As a result, the first 3-4 Principal Components seem to be the most useful for explaining the variance of the data. Any co-efficient below 0.3 was disregarded due to it not having a strong representation in the component. The

robustness of the data is described with the Kaiser Meyer-Olkin (KMO) score with a score above 0.5 showing that the data is robust and has relatedness. In data that had very little co-incidence either the KMO score would be low or the data would not rotate within 25 degrees of rotation. In relation to this thesis all PCA's would rotate within 8-11 iterations.

Principal Component	PC1	PC2	PC3
Dark arms			
Anterior Mantle Bar			
Posterior Mantle bar			
Median Mantle stripe			
Anterior Head Bar			
White landmark spots (white square)			
Mottle			
White Splotches			
White landmark spots (head)			
White Square			
White Head Bar			
Posterior transverse Mantle Bar			
Anterior Transverse Mantle Bar			

-1	-0.75	-0.5	-0.25	0	0.25	0.5	0.75	1

Figure 2.7: Components displayed as a matrix with loading(co-efficient) scores

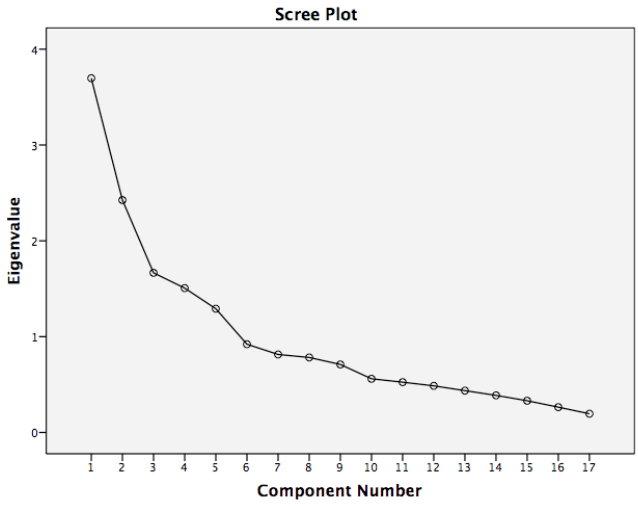


Figure 2.8: Example of Component Eigenvalue plotted against component number. Note the drop below 1 at component 6.

A result of doing PCA's is that the rotation of the data produces regression scores which allows plotting and investigation of the relatedness of the data to the Component matrix. With mean regression scores, ANOVA and post hoc tests can be performed to determine if there is a significant difference between components and their level of expression in different situations.

Chapter 3: Camouflage in complex visual environments

3.1 Introduction

3.1.1 Camouflage techniques in an aquatic environment

Endler (1978) suggested that cryptic camouflage can be understood as a sample of the background. Visual resemblance to the background decreases the risk of detection which is known as background matching (Merilaita and Tullberg 2005). The principle of being similar to the visual background with the colours and geometry of a prey colour pattern, the more difficult it should be for the predator to detect the prey (Cott 1940, Norris and Lowe 1964, Edmunds 1974, Endler 1978). It is also suggested (Endler 1978,1984) that colour patterning is cryptic if it resembles a random sample of the background in which they are most vulnerable. That is, in relation to time, age and microhabitat of the prey for visual predators. This theory also assumes all random samples of given background are equally cryptic (Endler 1978,1984). For cuttlefish, which can control their appearance, this raises the question of how they sample the background by integrating information over their visual field, and which background textures or objects drive the camouflage response.

3.1.2 Cuttlefish environment perception

The question of how cuttlefish sense 2-D backgrounds to produce a camouflage pattern has been well studied (Hanlon and Messenger 1988). When they settle on a flat substrate such as sand, gravel or a patterned surface they use a range of visual cues to select their pattern. These include the size of objects, the presence of edges, contrast in the pattern, and three dimensionalities of the substrate

(Kelman *et al* 2008; Zylinski *et al* 2009; Hanlon and Messenger 1988, Shohet *et al* 2006, Chiao *et al* 2009). However, the seafloor is not a continuous flat visual texture, but varies over short distances, and often includes larger objects such as stones and seaweed. This chapter examines how cuttlefish respond to features that do not form the substrate directly beneath the animal: namely seafloor backgrounds that are some distance away, and stones of varying size and contrast. In the latter case the cuttlefish must make two decisions: firstly, whether to settle near or far from the stone, and then whether to adopt the appearance of the stone, a strategy known as masquerade (Ruxton *et al* 2004; Skelhorn 2011) -, or to camouflage against the background. How do cuttlefish respond to such complex scenes, and what does this tell us about their perception and their camouflage behaviour?

Previously, in a study of asymmetrical body patterns, Langridge (2006) found that when cuttlefish settle with the left side of the body on one type of background and the right side on another they tend to adopt a symmetrical pattern that roughly averages the two backgrounds. Comparing vertical and horizontal patterns, Barbosa *et al* (2008) found that cuttlefish take both vertical and horizontal information into consideration when camouflaging. Ulmer *et al* (2013) demonstrated that vertical information is indeed integrated into overall camouflage decisions in *Sepia officinalis*, and, quite surprisingly, that information in the vertical field takes precedence over the horizontal information from the substrate. Buresch *et al* (2013) investigated the influence of 3D objects on cuttlefish camouflage, and focused on masquerade. They found that cuttlefish used masquerade rather than background matching in the presence of a nearby 3D object of high contrast, when the surrounding substrate is of low contrast.

Here we extend these studies by comparing how cuttlefish integrate information from across a horizontal visual field with the effects of a vertical ‘background’, and examine their responses to stones of

different sizes and contrast. As with other aspects of camouflage behaviour, we find that the animals integrate a range of visual cues to make complex behavioural decisions about the best defensive strategy in a given visual environment.

The aims of Chapter 3 are:

1.To record the behavioural response to the presence of varying stone contrast and sizes

2.Determine the effect of a 3-Dimensional stone characteristics of contrast and size on camouflage

3.To examine how distance of varying checkerboard backgrounds on a vertical and horizontal axis influence expression of camouflage

3.2 Methods

All experiments were conducted at Brighton Sea Life Centre in a designated filming tank. Cuttlefish were placed within a Perspex arena of 300mm diameter. We used 10 animals ranging from 120-360 days in age with 70mm-90mm mantle length. Following standard procedures (Kelman 2007), the expression of behavioural components by cuttlefish were blind selected and graded by eye from photographs on a three point scale with scores being entered into a SPSS v 22 spreadsheet. A Principal Component Analysis (PCA) with Varimax rotation was then conducted on the grading scores, and subsequent component regressions analysed by ANOVA and Bonferroni post hoc tests.

3.2.1 Experiment 1: High and low contrast objects on a natural background

To test responses to stones of varying size and contrast, cuttlefish were allowed to settle on a natural background of fine grade sand fixed with silicone to an acrylic sheet. Cuttlefish were then subjected to four different treatments (Figure 3.1). Stones of three sizes (30-50 mm, 70-100mm,150-170mm diameter) and three contrasts (Dark, Light, Medium) were placed in the centre of the arena (Figure 3.2).

Treatment (Stone colour)	Size	Reflectance (a.u)	Mean ratio mantle: stone size
Dark (Black)	small	0.26	1.81
	Medium	0.20	0.87
	Large	0.25	0.45
Light (White)	Small	0.68	1.52
	Medium	0.63	0.75
	Large	0.64	0.664
Medium (Neutral)	Small	0.34	2.01
	Medium	0.46	0.76
	Large	0.34	0.59
Sand		0.4	

Figure 3.1: Cuttlefish treatments divided by contrast and size of stone. Size of stone is divided into ratio small 1.5-2 cuttlefish times larger than the stone, Medium 0.75-0.9 stone similar size to the cuttlefish, Large 0.45-0.67 cuttlefish smaller than the stone. Sand with no stone was used as a control

Cuttlefish were allowed to settle for no less than 5 minutes and then filmed for 10 minutes. The film was then edited to 5 min lengths with photographic stills taken randomly from the film. The stills were compared first to make sure they were a fair representation of the overall film before the random selection. The distance the cuttlefish settled from the stone was also recorded.

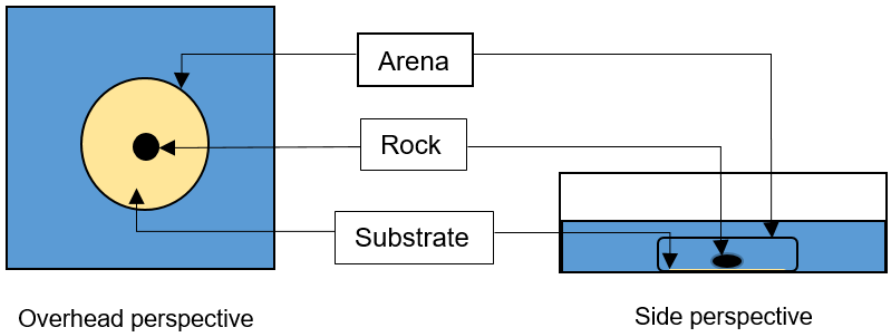


Figure 3.2: Experimental tank set up with ring enclosure. Sand substrate was used on the horizontal surface. Stone was positioned in the centre of enclosure

3.2.2 Experiment 2: Spatial Integration

Cuttlefish were placed in a smaller arena of 100mm diameter to stop movement over different backgrounds. A larger ring arena was also present at 300mm diameter for testing further vertical information. Three checkerboard sizes of small (3mm) medium (8mm) and Large (12mm) were selected (Figure 3.3).

Control	Half and Half	Outer Area Near	Outer Area Far	Vertical Near	Vertical Far
Small Check (S)	Small check/ Large check (S/L)		Medium check far (MF) Large check far (LF)	Small check near (SN)	Small check far (SF)
Medium Check (M)	Medium check/small check (M/S)	Small check near (SN) Large check near (LN)	Small check far (SF) Large check far (LF)	Medium check near (MN)	Medium check far (MF)
Large Check (L)	Medium check large check (M/L)	Small check near (SN) Medium check near (MN)	Small check far (SF) Medium check far (MF)	Large check near (LN)	Large check far (LF)

Figure 3.3: Checkerboard size divided between the outer area and inner area and vertical information.

These checkerboard designs were used either horizontally inside or outside of the arena or vertically in close proximity around the edge of the 100mm arena or further away on the 300mm area (Figure 3.4). The three checkerboard sizes were used inside the arena, and the outer area could be either the same as the inner arena or one of the other check sizes.

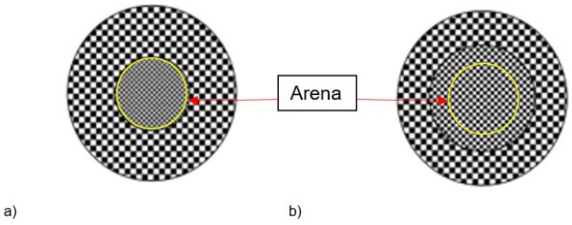


Figure 3.4: Examples of horizontal checkerboard set up (not to scale) a) inner circle small check outer circle medium check b) inner circle small check outer circle further away with middle check

3.3 Results

Experiment 1: High and low contrast objects on a natural background

Cuttlefish were placed in a relatively simple natural environment with one stone in the middle of the arena on a sand background. The stone varied in size and contrast. A sand substrate without a stone was the control.

3.3.1: Principal Component Analysis

The first three components explained 42 percent of the overall variance in the body coloration patterns. The Principal Components corresponded approximately to dark uniform (PC1), mottle (PC2) and disruptive (PC3) body patterns (Figure 3.5; Hanlon and Messenger 1988). The Principal Components were distinct, with only two features (Behavioural components) being shared by PC1 and PC3 (Figure 3.4)

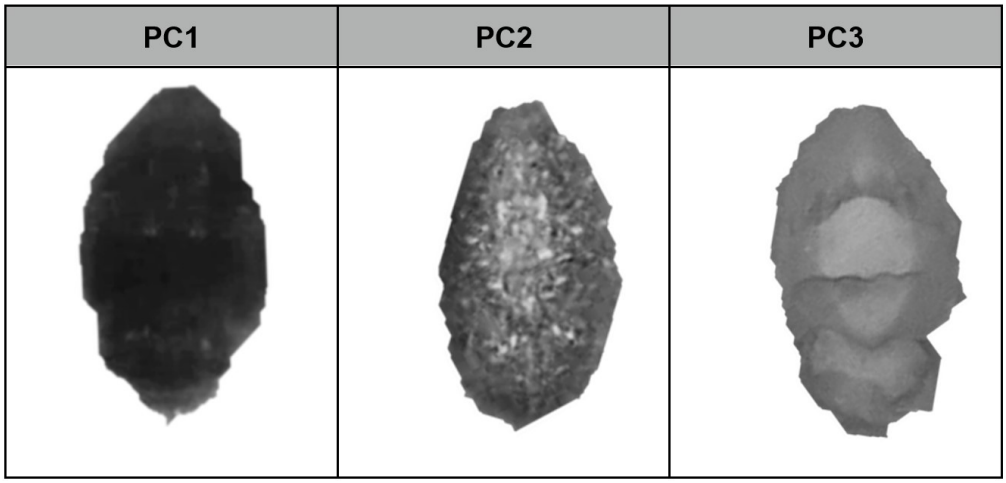


Figure 3.5: First 3 Principal Components explaining for 42 percent of the variance. Kaiser-Meyer-Olkin =0.755, p=0.0002

Principal Component	PC1	PC2	PC3
Dark Arms			
Anterior Mantle Bar			
Posterior Mantle Bar			
Median Mantle Stripe			
Anterior Head Bar			
White Landmark Spots (White Square)			
Mottle			
White Splotches			
White Landmark Spots (Head)			
White Square			
White Head Bar			
Posterior Transverse Mantle Bar			
Anterior Transverse Mantle Bar			

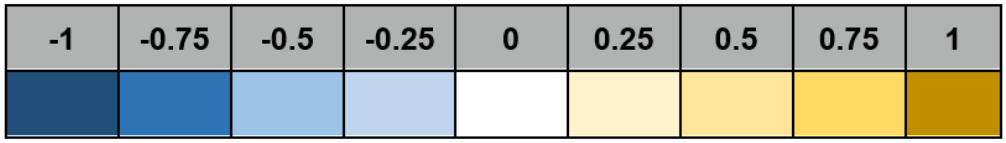


Figure 3.6: Principal Component Matrix for Experiment 1. All component scores were 0.5 or higher.

3.3.2 Location of cuttlefish in relation to stone

Cuttlefish are sensitive to both the size and the contrast of the stone in their choice of where to settle. They did not settle close to the small stone except with the medium contrast treatment. Settling by the stone increased as the stone size increased ($P<0.05$). (Figure 3.7). Cuttlefish chose to sit by the large stones in 75% of tests.

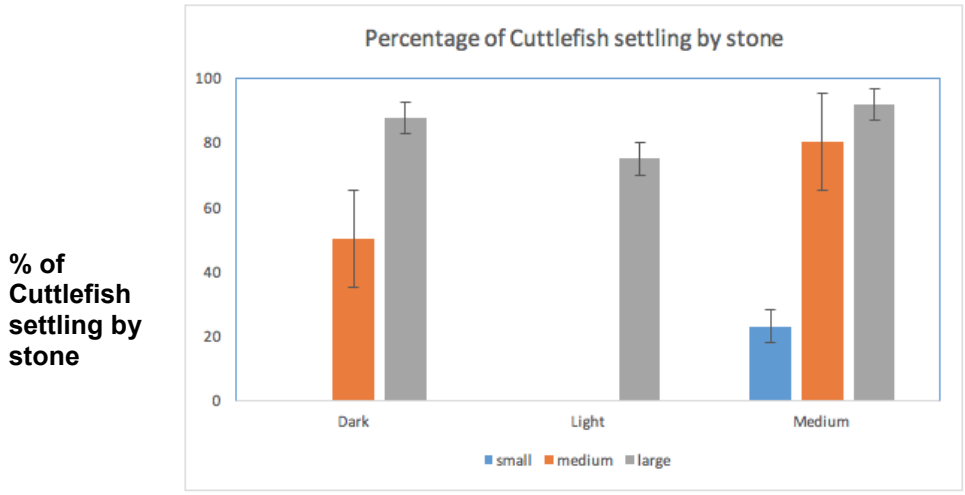


Figure 3.7: ***The choice to settle near a stone depends both its size and colour.*** **Percentage of cuttlefish located by the stone plotted against size of stone. Divided between the different colour treatments. Note that cuttlefish only chose to sit in all three sizes by the medium (neutral) stone and only the large stone in white**

3.3.3 Effect of location and stone contrast on cuttlefish colouration

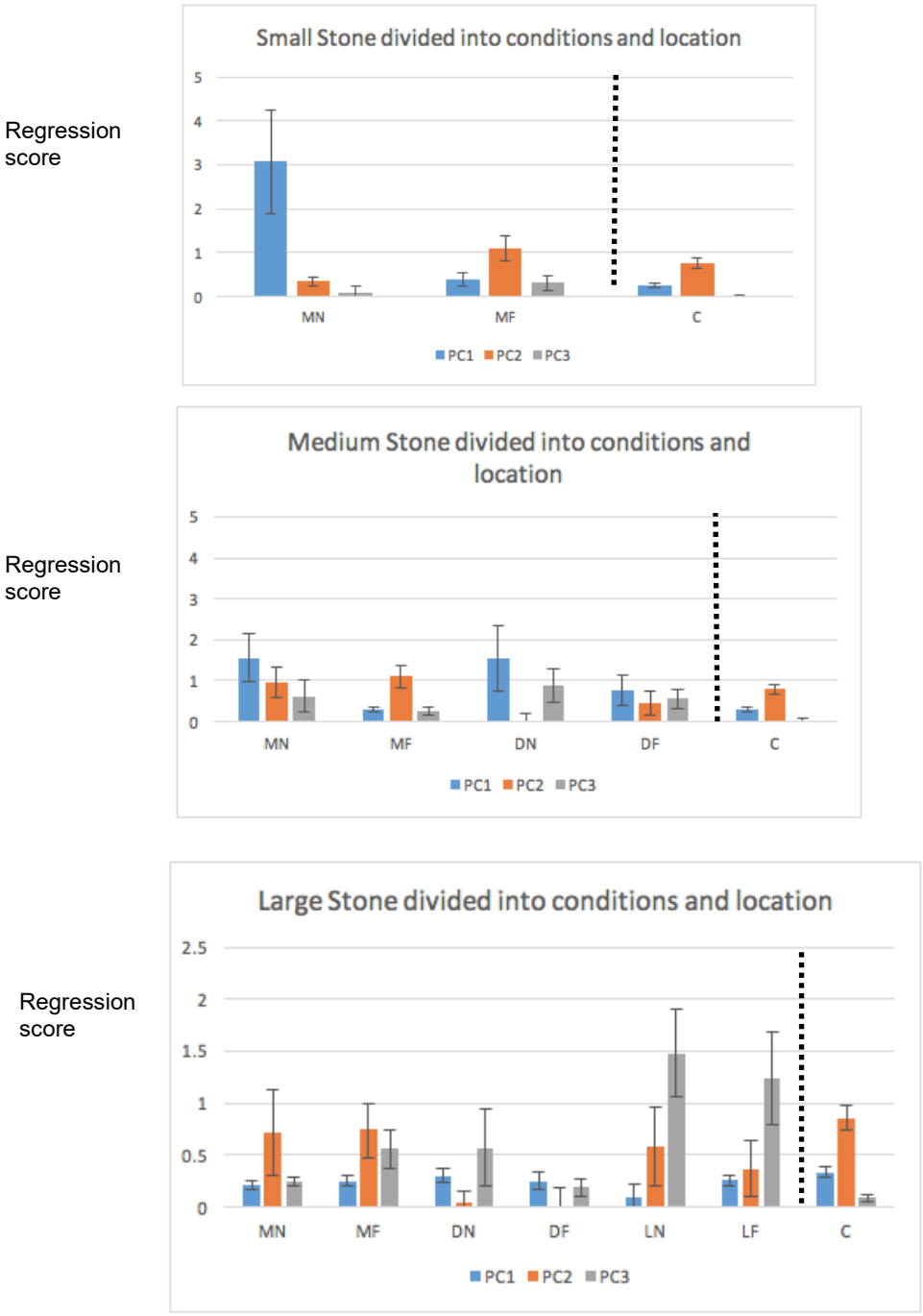


Figure 3.8: Effect of size, contrast and proximity of the stone on cuttlefish colouration. Expression of Principal Component scores divided into stone contrast groups. PC1 expression was significantly enhanced in the presence of dark and medium stones of medium and smaller size ($p<0.05$), and by proximity to the stone ($P<0.05$). PC3 was significantly greater ($p<0.05$) to the light stone than to the other two stone contrasts (DN=Dark stone Near, DF=Dark stone far, MN=Medium stone near, MF=Medium stone far, LN=Light stone Near, OF=Light stone far, C= sand).

Compared to the sand control, the presence of a stone affected the cuttlefish colouration pattern in varying degrees depending on whether the animal settled near or far from the stone (Figure 3.8).

Stone colour and size	Stone location	Response. Compared to sand control
Medium (30-50mm)	Near	PC1+
Medium (70-100mm)	Near	PC1+
Dark(30-50mm)	Near	PC1+
Dark (70-100mm)	Near	PC1+
Light (150-170mm)	Near	PC3+
Light (150-170mm)	Far	PC3+

Figure 3.9: Significant differences in response to sand control + designates a greater mean value. In Medium and Dark treatment PC1 was effected. In Light large stones PC3 was effected

The presence of the stone near the cuttlefish significantly ($p<0.05$) increased expression of PC1 (Uniform dark) in the dark(black) and Medium(Neutral) treatments with PC1 being significantly stronger ($p<0.05$) when the stone is near than when it is far (Figure 3.9). As the PC1 pattern resembles the stone this behaviour could be termed masquerade (Skelhorn *et al* 2011). The colour of the stones affected the expression of PC3 ($p<0.05$), mainly due to the increase with the size of the white stone (Figure 3.10). This Principal Component contained white components, and is consistent with previous studies (Chiao *et al* 2007). In contrast the medium (neutral) Principal Components became similar to the sand control in the large stone, and are not significantly different from each other ($p >0.05$.) suggesting that there is a different camouflage response to that of small or medium size medium (neutral) stone.

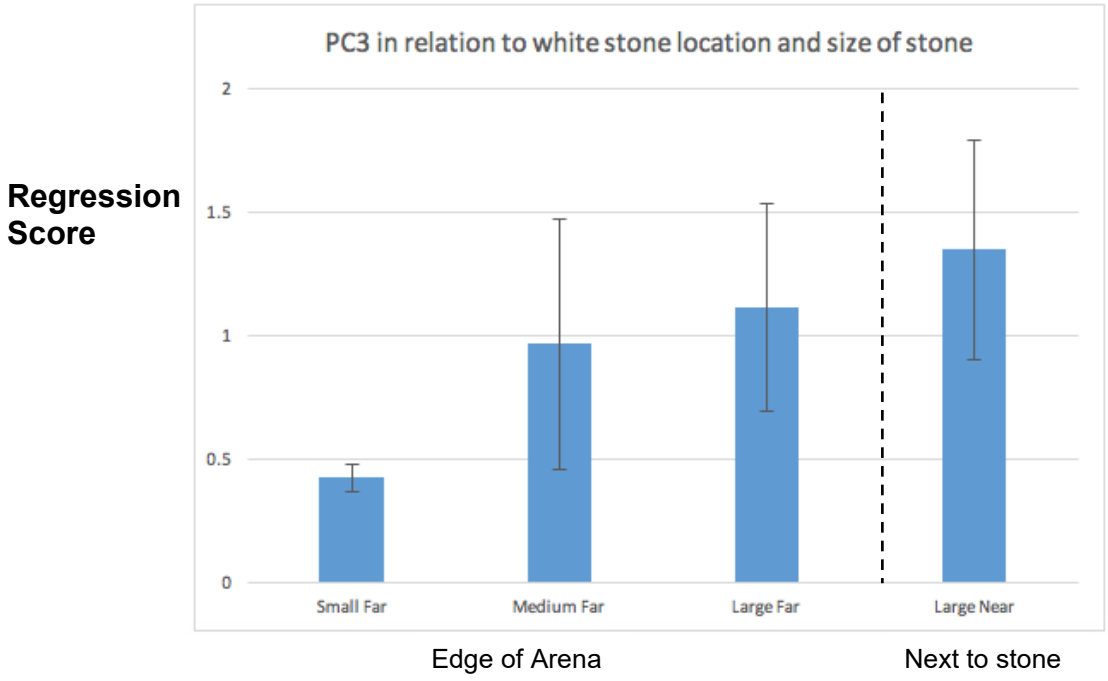


Figure 3.10: ***Cuttlefish only sat near white stones when they were larger than themselves*****. Graph of PC3 in relation to stone size and location in white stone treatment.**

Colour	Size	Near Stone	Far from Stone
Control			
Dark	Medium (70-100mm)		
	Large (150-170mm)		
Light	Large (150-170mm)		
Medium	Medium (70-100mm)		
	Large (150-170mm)		

Figure 3.11: Table of cuttlefish camouflage in relation to stone size and proximity to the stone.

Experiment 2: Spatial Integration

This experiment compared the responses of the animal to uniform horizontal backgrounds, with those backgrounds containing two visual textures (checkerboards), which were placed either horizontally or vertically. We also examined the effects of stimulus distance on these responses.

3.3.4 Principal Component Analysis

In this experiment, the Kaiser-Meyer-Olkin statistic of 0.69 three Principal Components explained 38% of the variance. PC1 and PC2 are disruptive patterns (though using mostly separate sets of behavioural components) (Fig. 3.12) with PC3 a mottle trait (Figure 3.12)

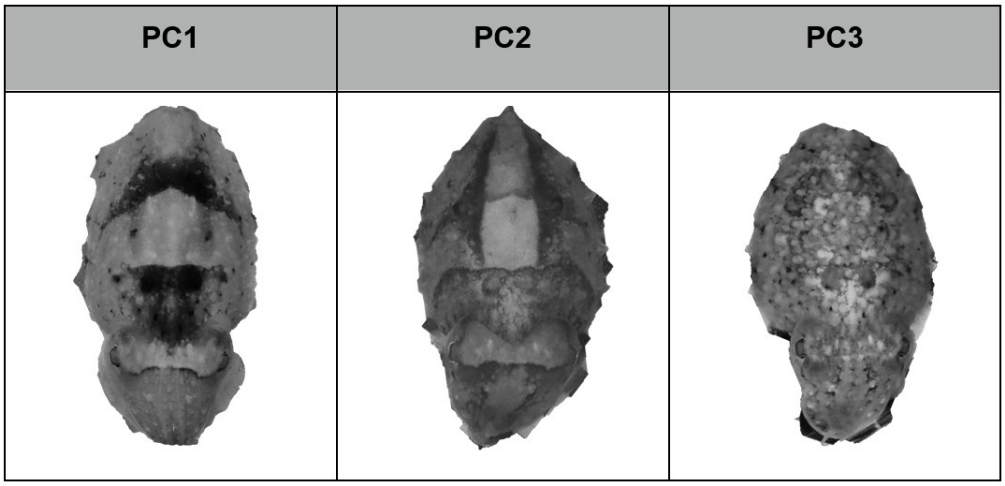


Figure 3.12: First 3 Principle Components explained 38% variance in the Principal Component Analysis. Kaiser-Meyer-Olkin =0.687, p=0.00

Principal Component	PC1	PC2	PC3
Posterior Transverse Mantle Bar			
Paired Mantle Spots (Anterior)			
Posterior Head Bar			
Anterior Transverse Mantle Bar			
Anterior Head Bar			
White Square			
Median Mantle Stripe			
White Posterior Triangle			
White Head Bar			
White Landmark Spots (White Square)			
White Splotches			
Mantle Margin Scalloping			
Paired Mantle Spots (Posterior)			
Cuttlebone			
White Mantle Bar			

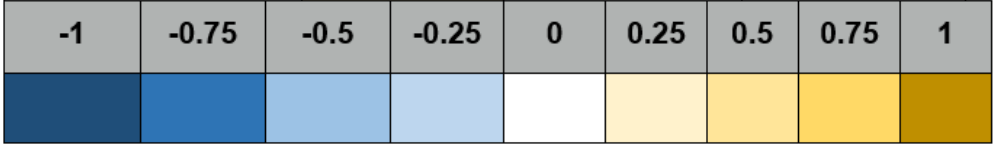


Figure 3.13: Matrix of first 3 principal components.

3.3.5 Horizontal checkerboards

Control checkerboards

As expected for a uniform checkerboard background, check size affected the body pattern (Figure 3.14; Zylinski *et al* 2009). PC1 and PC2 expression was significantly different ($p<0.05$) between the three checkerboard sizes suggesting that cuttlefish were expressing different camouflage on the different check sizes.

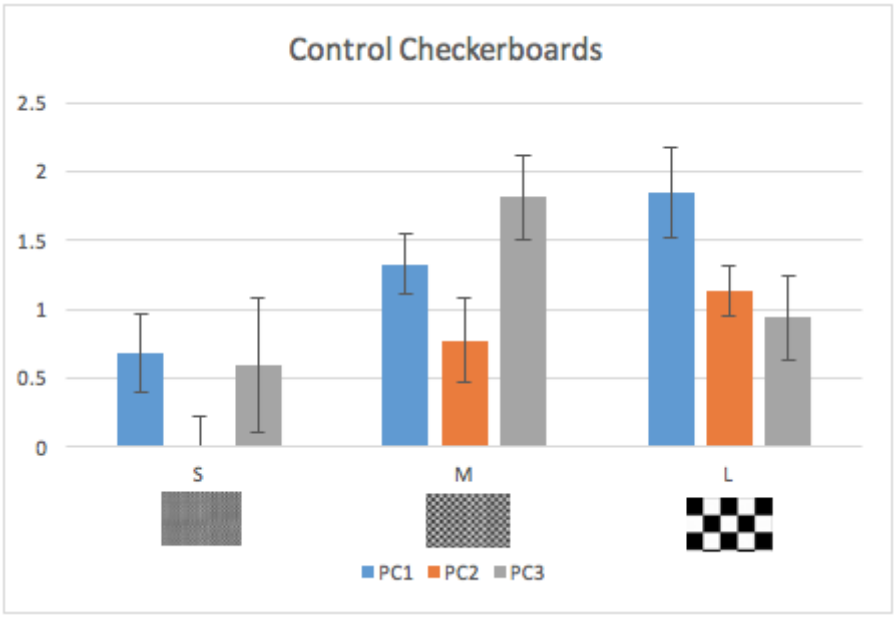


Figure 3.14: Control checkerboards on A4 sheets at 100% contrast. PC1 increases as the check size increases. S= Small Checkerboard, M= Medium Checkerboard, L= Large Checkerboard

Half and Half Checkerboard

When the cuttlefish rested simultaneously on two backgrounds PC1 showed the more variability than PC's 2,3. Medium/Large (M/L) mix and Small/Large (S/L) mix showed a significant difference in PC1 ($p<0.05$) (Figure 3.15). If we compare to the control (Figure 3.14) we can see a mixture of the control regression scores. PC1 showed the most variation against the control in the M/L treatments with PC1 showing a significant difference against the medium checkerboards, and PC2 significant difference against the large, suggesting that regression scores are dependent on the mixture of checkerboard sizes. The finding that cuttlefish lying on two backgrounds adopt an intermediate pattern is qualitatively consistent with previous work (Langridge 2006).

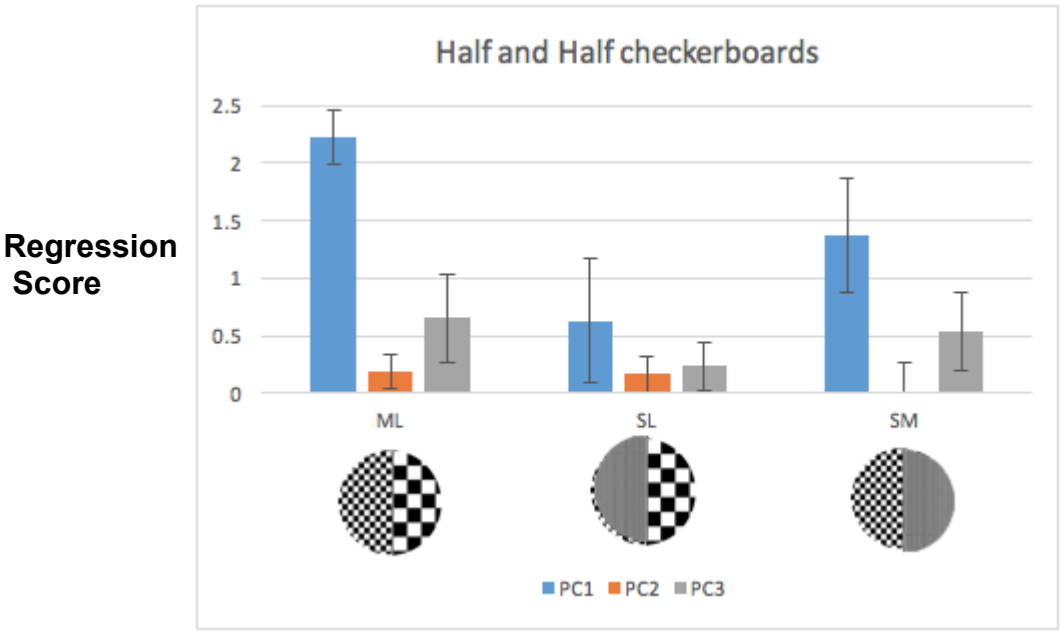
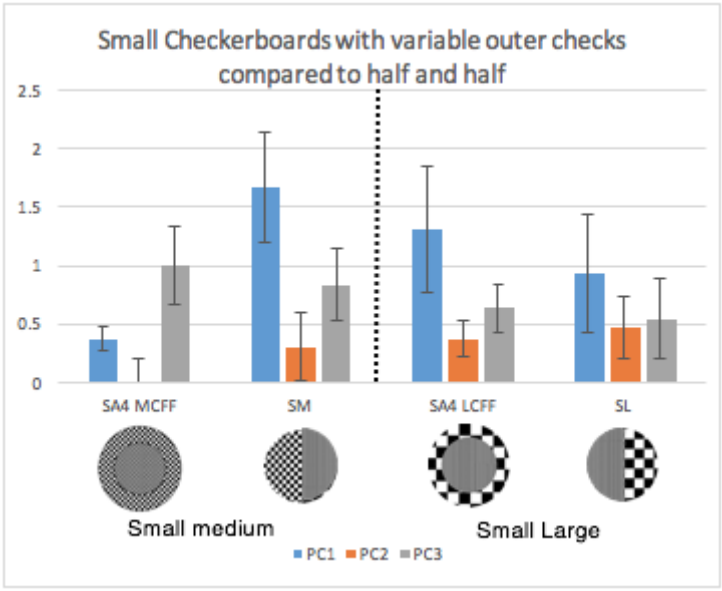


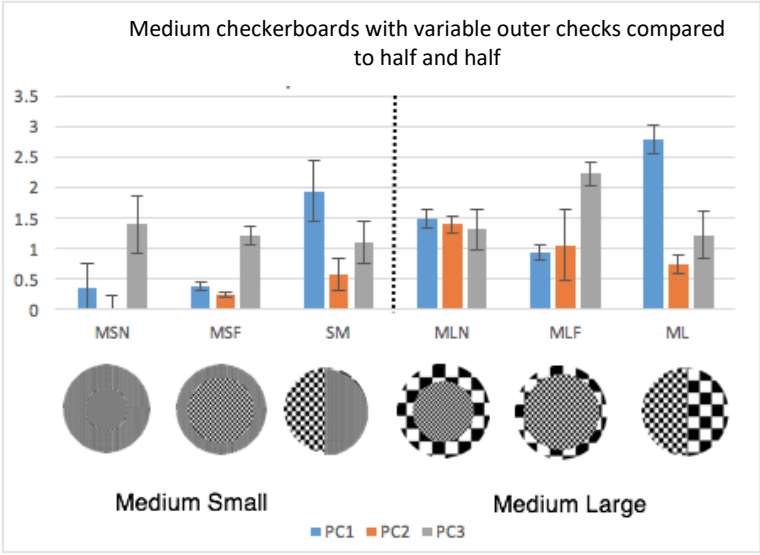
Figure 3.15: Half and Half Checkerboard at 100% Contrast. PC1 shows a significant difference between SL mix and ML mix ($p<0.05$). None of the half checkerboards showed similar component profiles. ML= Medium and Large mix, SL=Small and Large mix and MS= Medium Small

Horizontal checkerboards

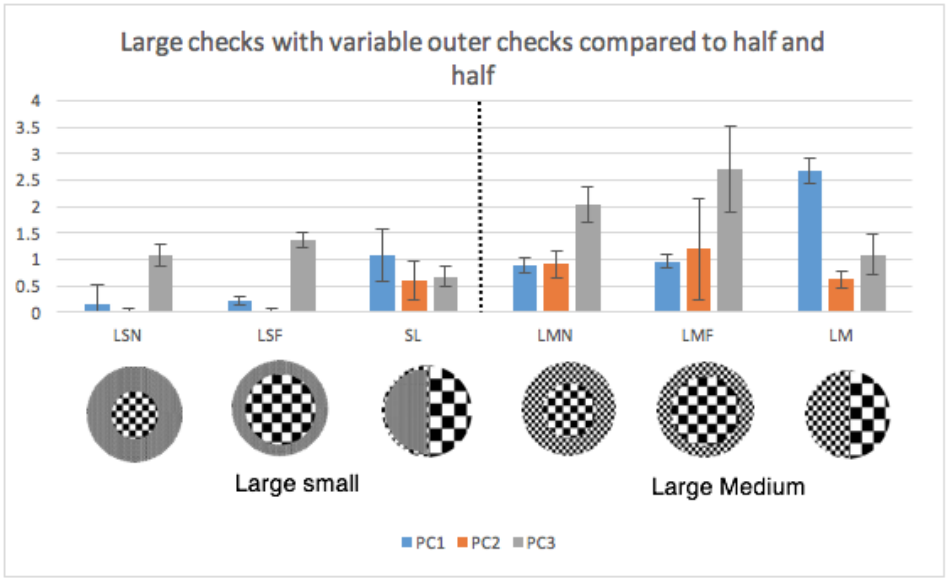
Small checkerboards showed similar component expression to that of the half and half checkerboards (P>0.05) (Figure 3.16a). In contrast PC1 in the medium checkerboards showed a diminished expression compared to the half and half in both sizes (P<0.05) (Figure 3.16b) suggesting that presence of another checkerboard had an effect although the distance did not seem to effect expression (p>0.05).



a)



b)



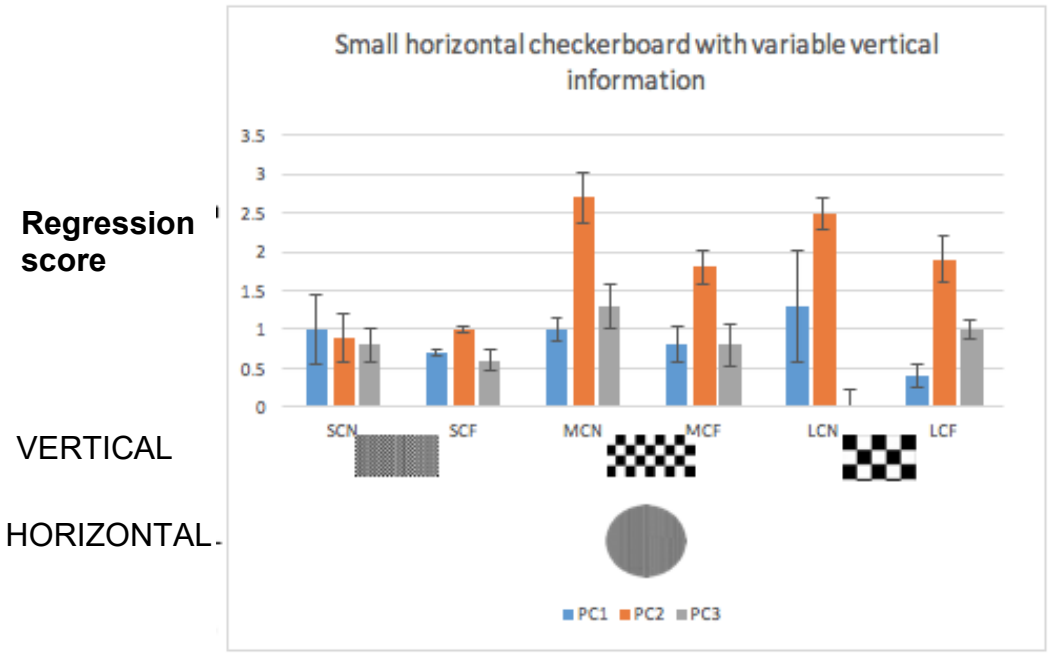
c)

Figure 3.16 : a) Small Check inner with medium and large check outer rim. SMF= Small check inner background Outer ring Medium check, SM=Small/Medium half and half check, SLF= Small check inner background Outer ring Large check, SL= Small/Large half and half check b) Medium Check inner Check with Small and Large check outer rim. MSN= Medium check inner background Outer ring Small Check near, MSF= Medium check inner background outer ring small check far SM=Small/Large half and half check MLN= Medium Check inner background Large check near, MLF= Medium Check inner background large check far c) Large check inner Check with Small and Large check outer rim. LSN= Large check inner background Small check near, LSF= Large check inner background small check far LMN= Large check inner background and medium check near, LMF= Large check inner background and medium check far

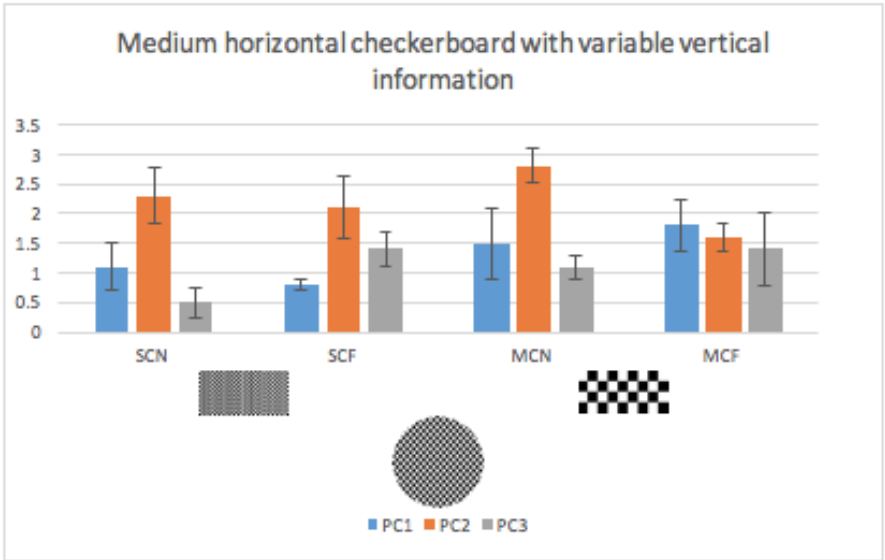
The response to the large checkerboard inner circle with small checks outer rim was not significantly different to the small/large half and half treatment, and showed similar levels of expression at both near and far small checkerboard (Figure 3.16c). PC1 was significantly different in large check inner circle medium near to that of the medium/large half and half. In both the large inner treatments, expression of PC's increased with distance. PC1 was the only component that showed significant variation in all the cases suggesting that this is most sensitive to checkerboard size for the horizontal patterns.

3.3.6 Vertical Checkerboards

Vertical distance information effects horizontal cuttlefish camouflage. In contrast to the horizontal checkboard information in which PC1 fluctuated in relation to treatment, PC2 showed the greatest variation and largest expression in all checkerboard sizes. In the small checkerboard PC2 was significantly different in the Medium and Large vertical to that of the small vertical checks both near and far ($p<0.05$).



a)



b)

Regression score

VERTICAL

HORIZONTAL

c)

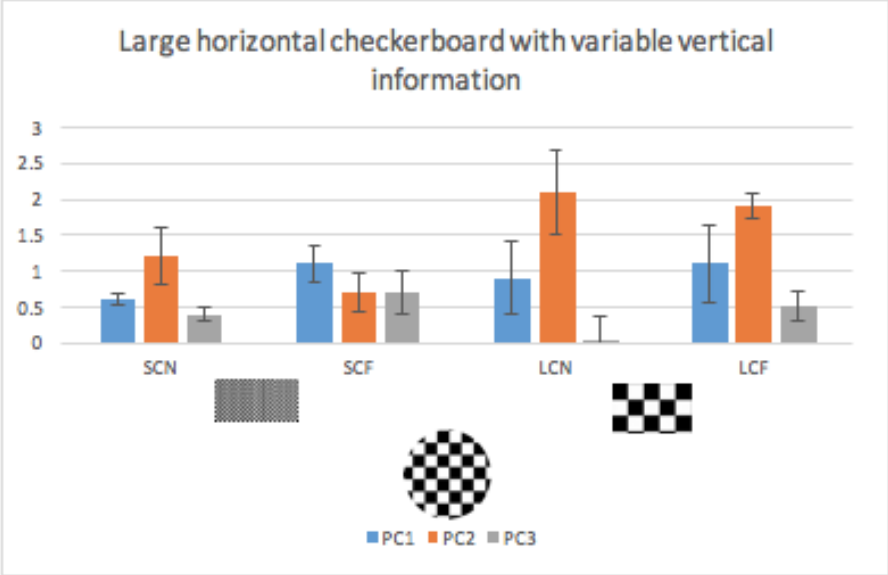


Figure 3.17:Checkerboard with varied vertical checkerboard information SCN= Small Checkerboard near, SCF= Small checkerboard far MCN=Medium Checkerboard near, MCF= Medium Checkerboard Far, LCN= Large Checkerboard near, LCF=Large Checkerboard Far a) Small checkerboard with small, medium and large vertical check at a close and far distance b) Medium checkerboard with small and medium check at close distance and far distance c) Large checkerboard with small and large check at close and far distance

In the medium checkerboard horizontal background, a small vertical checkerboard affected similar expression in both near and far conditions with a slight increase in PC3 at a greater distance (Figure 3.17b). With the vertical medium size checks PC2 decreased as distance increased, however not significantly. In comparison, the large checkerboard horizontal with vertical small checks showed a decrease in PC2 from that in the medium horizontal checkerboard. Again, PC3 increased in the far large vertical checks when distance increased (Figure 3.17c).

If we compare distance information of a vertical large checkerboard with that of a near vertical medium checkerboard we can see similarities in the expression of the Principal Components (Figure 3.18).

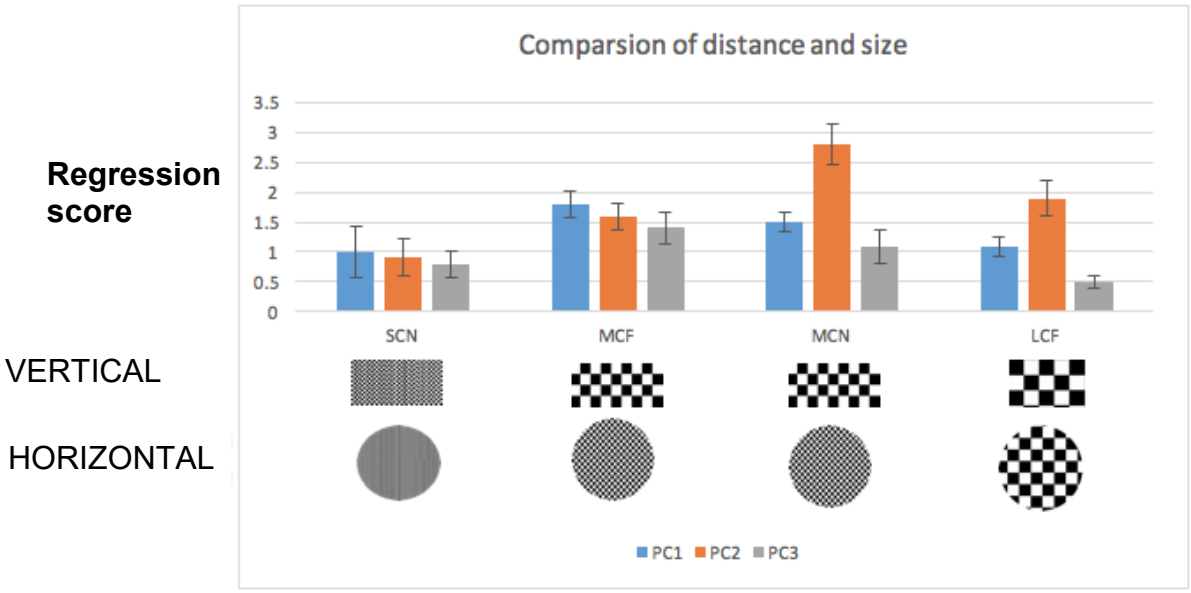


Figure 3.18: Comparison of distance and size of vertical information. Near information appears to have similar expression to larger size checks at a further distance SCN= Small check near, MCF=Medium check far, MCN= Medium check near, LCF= Large check Far

3.3.7 Overall Proximity Results

With the control checkerboards PC1 increased as size of checks increased and the presence of the white square can be seen in both the medium and the large checkerboards (Figure 3.14). When the cuttlefish were given half and half checkerboards they seem to produce a mixture of expression of patterns typical of the two checkerboard sizes similar to Langridge (2006). When the cuttlefish were surrounded by a different size checkerboard there seemed to be variation in their response. In the small checks the camouflage was similar to that of the half and half, however in the medium and large checkerboards PC1 was significantly different to that of the half and half treatments.

In the presence of a vertically oriented pattern the distance to the surface affected the expression of the body patterns. PC2 tended to a decrease with distance in all treatments, whereas PC3 increased with distance for both the medium and large checkerboards ($p<0.1$) In

general distance to vertical surfaces produced similar expression patterns to those typical of a smaller checkerboard nearby, suggesting that the animals responded to the size of the checks in the retinal image, rather than their real size. For example, the response to Large far check was like that of the Medium near checkerboard.

	Control			Half and Half		
Control						
	Small	*Medium*	*Large*	*Small/Medium*	*Small/large*	*Medium/large*
Near						
	MSN	*MLN*	*LSN*	*LMN*		
Far						
	MSF	*MLF*	*LSF*	*LMF*	*SMF*	*SLF*
Vertical						
	SCN	*SCF*	*MCN*	*MCF*	*LCN*	*LCF*

Figure 3.16: Representation of camouflage in each of the different treatments.

3.4 Discussion

3.4.1 Behaviour response to location of stone

Cuttlefish commonly chose to settle near to vertical surfaces, such as aquarium walls, while Buresch *et al* (2011) concluded that *S.officinalis* prefer to resemble a 3D object than the benthic substrate. Here I have shown that these characterizations are nuanced, with the preference to settle near the stone and the choice of whether to masquerade or to match the background being dependent on the size and contrast of the stone. Experiment 1 finds that this behaviour depends upon the size and the contrast of the object. The likelihood that cuttlefish would settle adjacent to a stone increased with its size over the range of 0.5 – 2 mantle lengths, and were also affected by its contrast. Cuttlefish only settled near stones of the smallest size when they were Medium contrast(Contrast 0.36); at least 50 % of cuttlefish chose to settle beside medium sized stones when they were medium or dark stone, but 0% when they were light. For the largest size of stone 75% of the cuttlefish chose to settle by the stone. The reasons for this selectivity are not clear. It may be that the stone offered little protection or drew attention to the location of the cuttlefish at smaller sizes, as the cuttlefish can vary their colour from light to dark it is unclear why contrast had such a clear affect. If we compare these results to that described in octopuses we can draw some similarities. Josef *et al* (2012) determined that both *Octopus cyanae* and *Octopus vulgaris* based their body patterns on selected features of nearby objects. It was suggested that this allows the octopus to camouflage in partly occluded environments.

3.4.2 Effect of contrast and size

In addition to making a choice about whether to settle next to a stone or further way the cuttlefish can either use masquerade to resemble the stone, or match the background. Here we can elaborate on this finding. When settled by the stone, expression a disruptive camouflage increased as stone size increased, whereas at the edges camouflage was more uniform light components and close to the sand control *(p>0.05*). Disruptive patterns were shown with larger light stones, consistent with Chiao *et al* (2007) and other's finding that cuttlefish react to white cues as well as to the spatial scale and overall brightness of the background. The best evidence for masquerade camouflage is seen in the responses to the dark and medium stones, where a uniform almost black camouflage was displayed.

If we consider the viewpoint of a potential predator dependent on the angle of their viewpoint cuttlefish could be perceived as an extension of an object or another rock (Endler 1991). Possibly the key factor in causing the masquerade is that the natural background was uniform which could potentially draw greater detection of the cuttlefish's outline. Ruxton *et al* (2004) suggests that masquerade sometimes involves a close evolutionary relationship. Stuart-Fox *et al* (2008) suggested that dwarf chameleons may be able to adjust their camouflage in relation to differences in predator visual systems. This raises the question is there predatory pressure driving the masquerade? Visibility in the habitat in which *S.officinalis* lives in, is variable sometimes with low visibility. Predators in a low visibility environment may use different techniques other than vision to detect prey, or certain aspects of vision for detection such as outline detection or motion. In this case masquerading as a rock would be a good technique for the cuttlefish to avoid detection.

3.4.3 Horizontal proximity checkerboards

Allen *et al* (2010) suggested that cuttlefish take visual cues from their surroundings and respond with the appropriate camouflage in addition to the background directly beneath the animal. Consistent with this observation we have found that in addition several factors affect the choice of camouflage. They integrate information over a horizontal range exceeding a body length, and take account of vertical and 3D information in their visual fields. Distance of this pattern significantly effects camouflage expression.

In a situation where the cuttlefish was restricted in its choice of location, we can see that the cuttlefish take multiple cues horizontally, as described by Langridge (2006) and Allen *et al* (2010). When presented with backgrounds of differing check size on either half, cuttlefish combined cues to a mixture between the two check sizes. When the same information was presented with the differing check size in an outer ring cuttlefish exhibited similar combination of camouflage, rather than displaying the camouflage for the background that they were settled on. The camouflage exhibited was more disruptive in its expression with PC1 and PC3 being significantly different when there was different check size horizontally in the distance. When the outer ring of the check size was moved even further away similar results were found, although slightly diminished, suggesting that there is some component of size being been detected independent of distance.

3.4.4 Vertical proximity checkerboards

Ulmer *et al* (2013) suggested cuttlefish respond to small percentage of their visual field and that vertical information plays a key visual feature to drive camouflage. Whereas, Allen *et al* (2010) showed cuttlefish integrated conflicting visual cues by producing mixed body patterns

similar to what was seen in this study on a horizontal field. Cuttlefish when presented with camouflage in the vertical field seemed to follow more Ulmer *et al* (2013) results and responded to the vertical information and similar expression of the patterns of the PC's, even if the horizontal checkerboard pattern was different rather than a combination of both.

3.4.5 Comparison of Horizontal and vertical checkerboards

Behaviour as well as the visual aspects of the environment seem to play an important role in overall camouflage decisions. When sat in proximity of objects monocular cues such as visual angles, textures, size and motion parallax may factor into cuttlefish visual processing. Vertical and horizontal information feed as 3D information into a 2D image on the retina in vertebrates, and then convert back into 3D information to allow for judging of the environment. Josef *et al* (2014) investigated if this held true for cuttlefish by looking at visual textual density gradient. They found that cuttlefish sensed illusory crevasses created by a visual texture density gradient. As texture density gradient relates to change in size and shape that occur as a function of depth or distance, we can relate this back to the results exhibited with the proximity of checkerboards. The fact they combined camouflage similar to the half and half checkerboards on the horizontal field, would suggest that cuttlefish have some ability to determine depth cues and differentiate size of objects in their visual field.

3.5 Conclusion

Cuttlefish moving and locating themselves near objects would gain protection along with possible masquerade camouflage. Cuttlefish visual ability and the flexibility of their behavioural responses allows them to protect themselves in the visually varied environment they inhabit. Integrating over an area of the substrate well beyond their immediate background and making nuanced – and to us inexplicable - choices about whether to use masquerade. Oddly however, they do not seem to take account of distance in ‘matching’ vertical surfaces.

Chapter 4: Effects of viewing conditions on camouflage

4.1 Introduction

As explained at the start of Chapter 3, it is usual to assume that camouflage patterns will tend to match, or perhaps to be suited to the background. However, a pattern is very unlikely to exactly match a background that is not of a uniform colour. In fact, it may be the visual information the animal can use to sense the background, and more important that a predator can use to break the camouflage, will depend upon the viewing conditions. For example, Zylinski (2009) found that in good viewing conditions cuttlefish are sensitive to components in checkerboard with a spatial period exceeding about 4mm. One might expect (Land and Nilsson 2012) that the sensitivity of both the cuttlefish and visual predators will be adversely affected by reducing the light level, or increasing the turbidity of the water. Nonetheless a recent study (Allen *et al* 2010) concluded that cuttlefish camouflage is unaffected by light level down to intensities close to starlight – when human spatial resolution is severely compromised (Land and Nilsson 2012). This chapter investigates this question further, and finds that both light level and turbidity strongly affect patterns in a way that is not consistent simply with a reduction in visual contrast. We cannot however be sure whether the cuttlefish is 'misreading' the background, or is seeing it correctly but making adjustments to take account of what a fish at some distance from the animal would see.

4.1.1 Optical properties of saltwater

Factors such as turbidity of the water, light conditions and shadow are found in the aquatic environment and potentially influence camouflage. Chemical changes in the water such as eutrophication and chemical

pollution can lead to algal blooms. this in addition to mechanical disturbance such as churning of substrate and the suspension of inorganic particles causes disruption to light propagation (Johnsen 2012). In addition, turbidity adds to light absorbance of pure water to reduce the overall amount of ambient light in the water column. Light in turbid water is therefore more scattered, and depending on depth, can change the perceived colour (Ender 1990). This can alter an organism's visual capability in the environment.

Signal to noise ratio is an important measure of how well a visual system can see under low light, and is an effective way to determine the ability to distinguish two objects of similar radiances (Johnsen 2012). This can then be observed in the context of a predator prey interaction and camouflage that may result in hiding from a predator in a low visibility environment. Different levels of light promote various levels of counter shading. Where light intensity is higher, countershading may be more prevalent than in situations where scattering is also high (Hailman 1977). When particles scatter light a brighter veil is seen between the object and the eye. This in turn degrades the object brightness and colour contrast when hitting the individuals' eye (Lythgoe 1979). This loss of information to an organism such as a fish predator could be costly in terms of finding prey, as enough light must be reflected from a sufficiently large surface and sufficient visual contrast to distinguish it from the background (Utne-Palm 2002). In fish the probability of prey detection has been found to be proportional to the reaction distance of the fish (Confer and Blades 1975). Low light and turbidity severely hinder this, although fish may deploy additional senses like juvenile Atlantic cod, which use olfaction for prey detection (Meager and Batty 2007).

Scattered light is polarised (Johnsen 2012), which means animals such as cuttlefish that are able to detect polarised light may use this ability to enhance contrast in turbid environments. This, in addition to their ability to vary camouflage so successfully in a variable ocean

environment, makes them an interesting organism to study in low light and turbid environments.

4.1.2 Cuttlefish camouflage in reduced visibility water

Cartron *et al* (2013) looked specifically at polarised reactions in the presence of a predator in both clear and turbid environments. The cuttlefish (*Sepia prashadi* and *Sepia pharaonis*) however, were not tested so much for their camouflaging in relation to the turbidity, but to the presence of a predator in a turbid environment. It was suggested the large size, sudden increase of predator's shape aided in identification of the threat in a turbid environment. As *S. officinalis* live in a coastal environment which is highly variable in light conditions, it is important to test how cuttlefish react to these conditions without a predator stimulant. Therefore, it is hoped set of experiments will help to achieve a better understanding on how cuttlefish camouflage relates to natural environment, without a sudden stimulus.

Previous studies by Allen *et al* (2010) showed cuttlefish continued to camouflage under low light conditions. Whether this was to deceive predators or prey was not determined as the study focused on whether the cuttlefish could camouflage themselves in such low light conditions. It was suggested that maintaining camouflage may be energetically costly, and if it was not necessary they would not express any camouflage. A uniform pale colour due to all chromatophores being closed (Messenger 2001) is the lowest energy state of the animal. Allen *et al* (2010) found cuttlefish do indeed camouflage under low light conditions. The question is the camouflage maintained due to predator/prey pressure, reaction to the information received by the eye, or a conscious decision of the cuttlefish. If it is a reaction to the reduced information to the eye then by testing it in reduced light conditions and high turbidity conditions against low contrast we may see similar responses.

In this experiment cuttlefish were tested in relation to varying strengths of turbidity to see how scatter of light effects their camouflage response. In contrast varying light conditions were also tested to determine if there were any similarities in response of camouflage. The results from these conditions where then compared with low contrast responses to investigate if they were of a similar response.

The aims of Chapter 4 are:

1. To determine the role turbidity has on cuttlefish camouflage

2. To investigate how low light effects camouflage

3. To compare any similarities of camouflage between lowlight and turbidity and that of low contrast

4.2 Method

All experiments were conducted at Brighton Sea Life centre in a designated filming tank. 10 animals were used, ranging from 120-360 days in age. As for Chapter 3, cuttlefish images were graded by eye on a three point scale with scores being entered into a SPSS v22 spreadsheet. A principal Component Analysis (PCA) was then conducted on the grading scores and subsequent component regressions analysed by ANOVA.

4.2.1 Experiment 1: Turbidity on checkerboard backgrounds

Three different checkerboards of 100% contrast (0.93 a.u) and varying sizes (check size 30mm, 80mm and 120mm) were placed in the main filming tank. Cuttlefish were housed in a separate circular tank (160mm diameter) within the main filming tank of (900 x 750mm). To make the water turbid 100, 200 and 400ml of oat milk was mixed into 100 Litres of saltwater in the outer part of the tank (Figure 4.1). The mixture was then left for 10 minutes to acclimatise in tank. Cuttlefish were photographed every minute for 5 minutes, and an image randomly selected from the five for analysis. Cuttlefish appeared to have no adverse reaction to the oat milk, which resembles suspended organic matter that might be found in many coastal habitats.

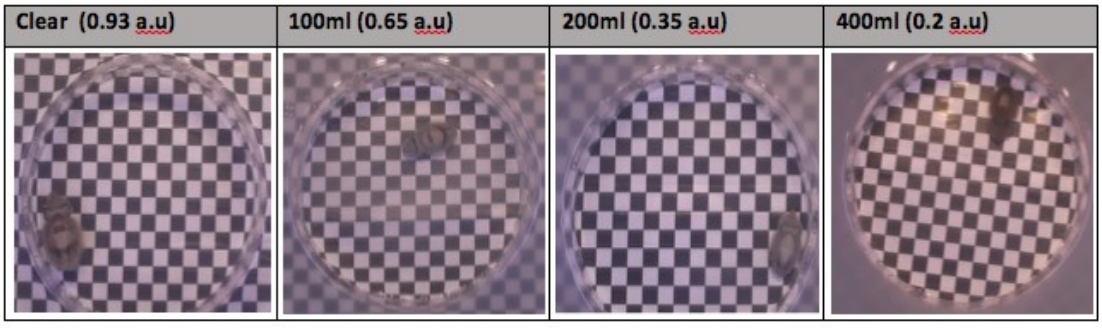


Figure 4.1 Different concentrations of oat milk in 100 litres of see water

Calibration of scatter in turbid media is complicated (Johnsen 2012), and here we simply measured the degradation of the image as it was

transmitted through the turbulent medium. This was done by looking at the modulation transfer function (MTF) of images passing through the 200mm depth of the tank. Photographs of a graduated scale in the different concentrations were taken and the Michelson coefficient calculated at numbers of line visible (Figure 4.2).

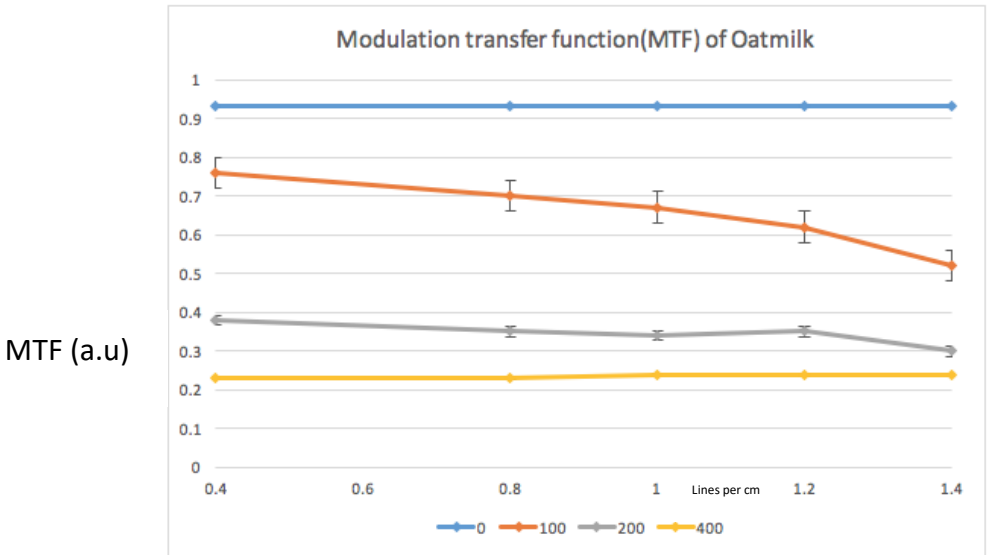


Figure 4.2: Modulation transfer function of 200mm of oatmilk represented by the contrast of black and white gratings of varying width viewed through 200mm with the oatmilk concentrations used in this study. The insensitivity to grating size suggests that scattered light had the main affect.

4.2.2 Experiment 2: Low light with checkerboard backgrounds

Cuttlefish were tested on three different sized 100% contrast checkerboard backgrounds (30mm,80mm and 120mm) at three different illuminations (10000lux, 1000lux and 0.1lux), which correspond roughly to light levels experience under bright sunlight, overcast daylight and moonlight (Land and Nilsson 2012). Cuttlefish were photographed every minute for 5 minutes and an image randomly selected from the five to analyse.

4.2.3 Control for contrast

To test whether the effects of varying light level and turbidity were equivalent to changing the contrast of the background, the animals were also tested on backgrounds of 100% (nominal), 50%, 25% and 0% contrast.

4.3 Results

4.3.1 Principal Component Analysis

Principal Component	PC1	PC2	PC3
White Square			
Anterior Transverse Mantle Bar			
Anterior Head Bar			
Paired Mantle Spots Anterior			
White Head Bar			
Paired Mantle Spots Posterior			
Posterior Head Bar			
White Landmark Spots (Arms)			
White Landmark Spots (Head)			
White Landmark Spots (White Square)			
White Splotch			
Dark 1st Arms			
Posterior Transverse Mantle Bar			
Median Mantle Stripe			
Mantle Margin Scalloping			
White Major Lateral Papillae			
White Mantle Triangle			
Anterior Mantle Bar			
Dark Arms			
Posterior Mantle Bar			
White Mantle Bar			

-1	-0.75	-0.5	-0.25	0	0.25	0.5	0.75	1

Figure 4.3: Principle Component matrix for combined turbidity, low light conditions and reduced contrast with values greater than 0.3 values. KMO score was 0.66 and 50% of the variance was explained in the first three factors.

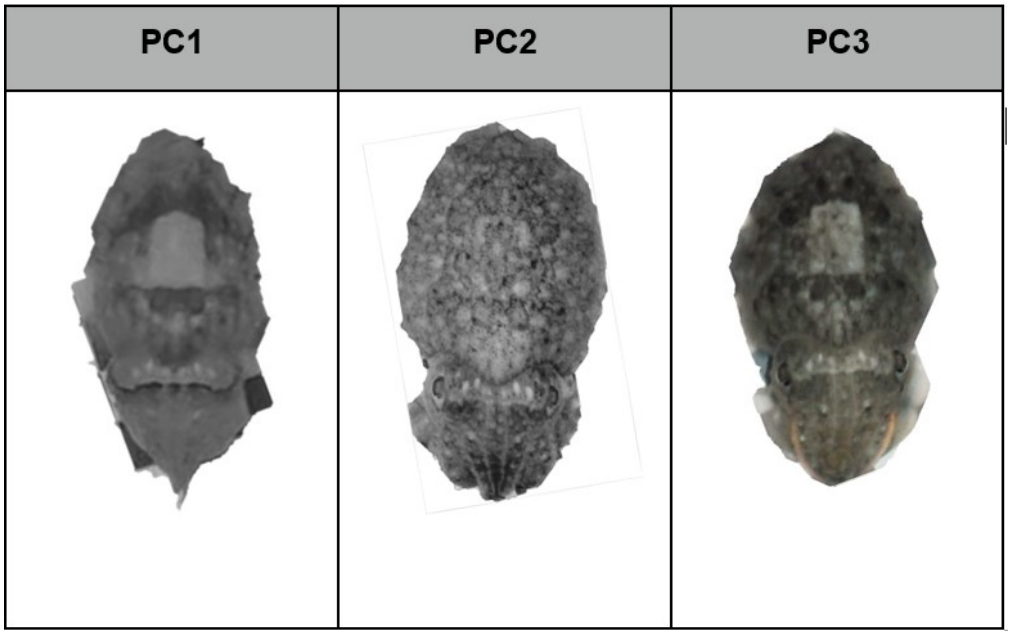


Figure 4.4: First 3 expressed Principal Components, PC1: disruptive characteristics PC2: mottle characteristics, PC3 A mixture of PC1 and PC2

Principal Component 1 (PC1) was more disruptive in its camouflage patterning with a slight mottle with Principal Component 2 (PC2) being a more mottled effect going towards uniform, and Principal Component 3 (PC3) a mixture between PC1 and PC2 with lighter components (Figure 4.4). Here we will discuss the PC1 and PC2 components as PC3 showed no significant difference between treatments ($p>0.05$).

4.3.2 Experiment 1: Turbidity on checkerboard backgrounds

As expected there was a strong effect of checkerboard size ($p<0.05$) on the coloration pattern. In addition to differences between checkerboards, as turbidity increases PC1, which has disruptive characteristics, decreased ($p<0.05$). In contrast PC2 which has a more mottled appearance, increased with increased turbidity ($p<0.05$). (Figure 4.5)

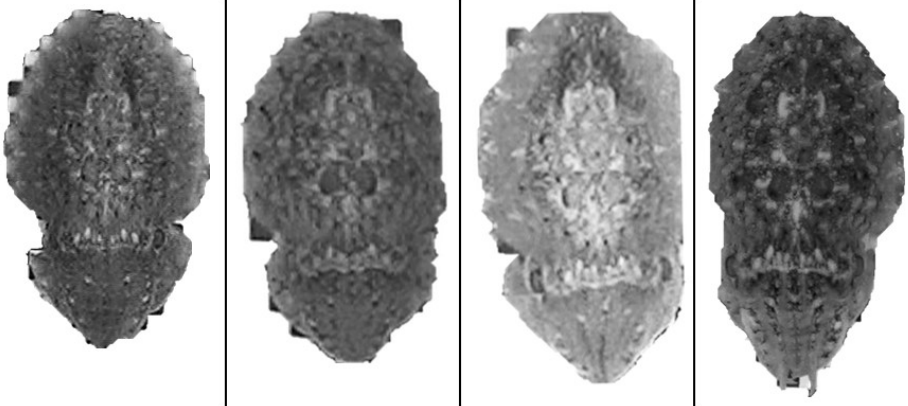
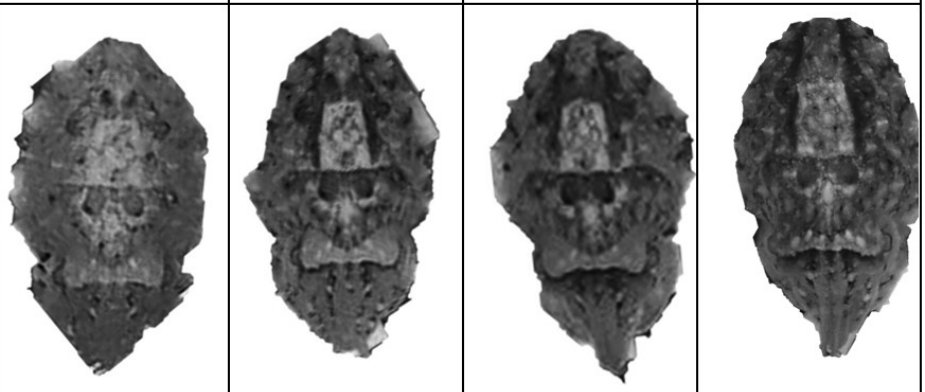
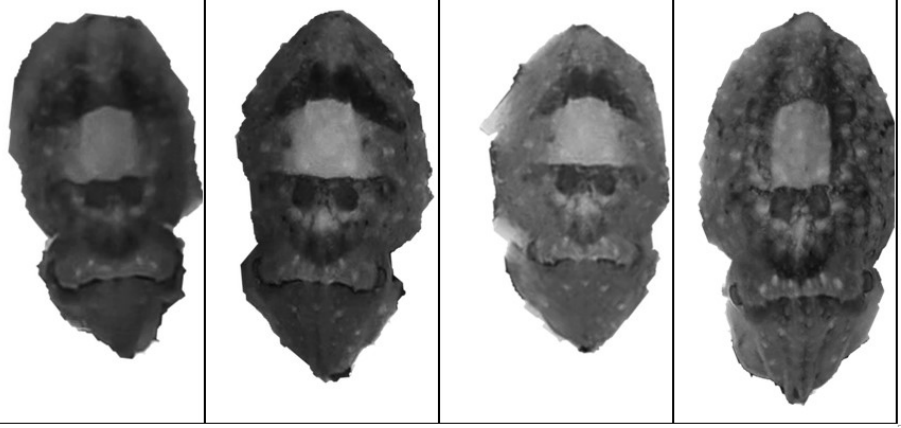
Size	0ml (0.95 a.u)	100ml (0.65 a.u)	200ml (0.35 a.u)	400ml (0.2 a.u)
Small				
Medium				
Large				

Figure 4.5: Table of turbidity camouflage categorised into intensity and checkerboard size. As turbidity increases white papillae increase and darker components decrease.

4.3.3 Experiment 2: Low light on checkerboard backgrounds

PC2 shows a significant difference ($p<0.05$) when check size and light intensity are accounted for. Figure 4.6 shows that as light is reduced a more mottled effect is seen in the cuttlefish, which is part of the component of PC2 (Figure 4.1). PC1 showed no significant difference in intensity and check size with interaction.

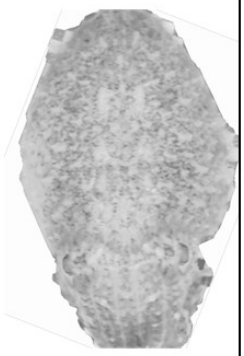
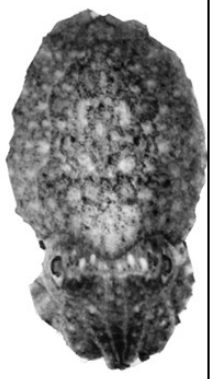
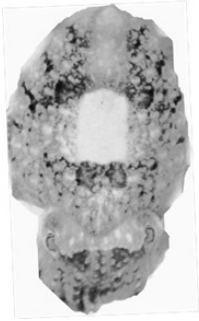
Size of Check	Full Light (10000lux)	1/2 Light (1000 lux)	Dark (0.1 lux)
Small			
Medium			
Large			

Figure 4.6: Table of light intensity versus checkerboard size

Comparison of the maximum turbid water concentration and the darkest condition yield similar responses, with increased areas of white patches and papillae (Figure 4.7), which were absent on reduced contrast backgrounds.

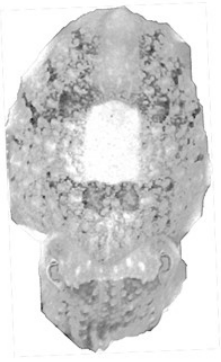
Size	High Turbidity (0.2 a.u)	Low Light (0.1 Lux)
Small		
Medium		
Large		

Figure 4.7: Comparison between the lowest light and highest turbid environment broken down into checkerboard size

When we compare the turbid, low light and low contrast, there are similarities in the expression of the PC's when comparing the low light and high turbidity ($p>0.05$), (Figure 4.8), and clear differences from the reduced contrast backgrounds ($P<0.05$). Hence not only does turbidity and low light seem to have an effect on camouflage as it increases but, it is not simply equivalent to varying contrast ($p<0.05$).

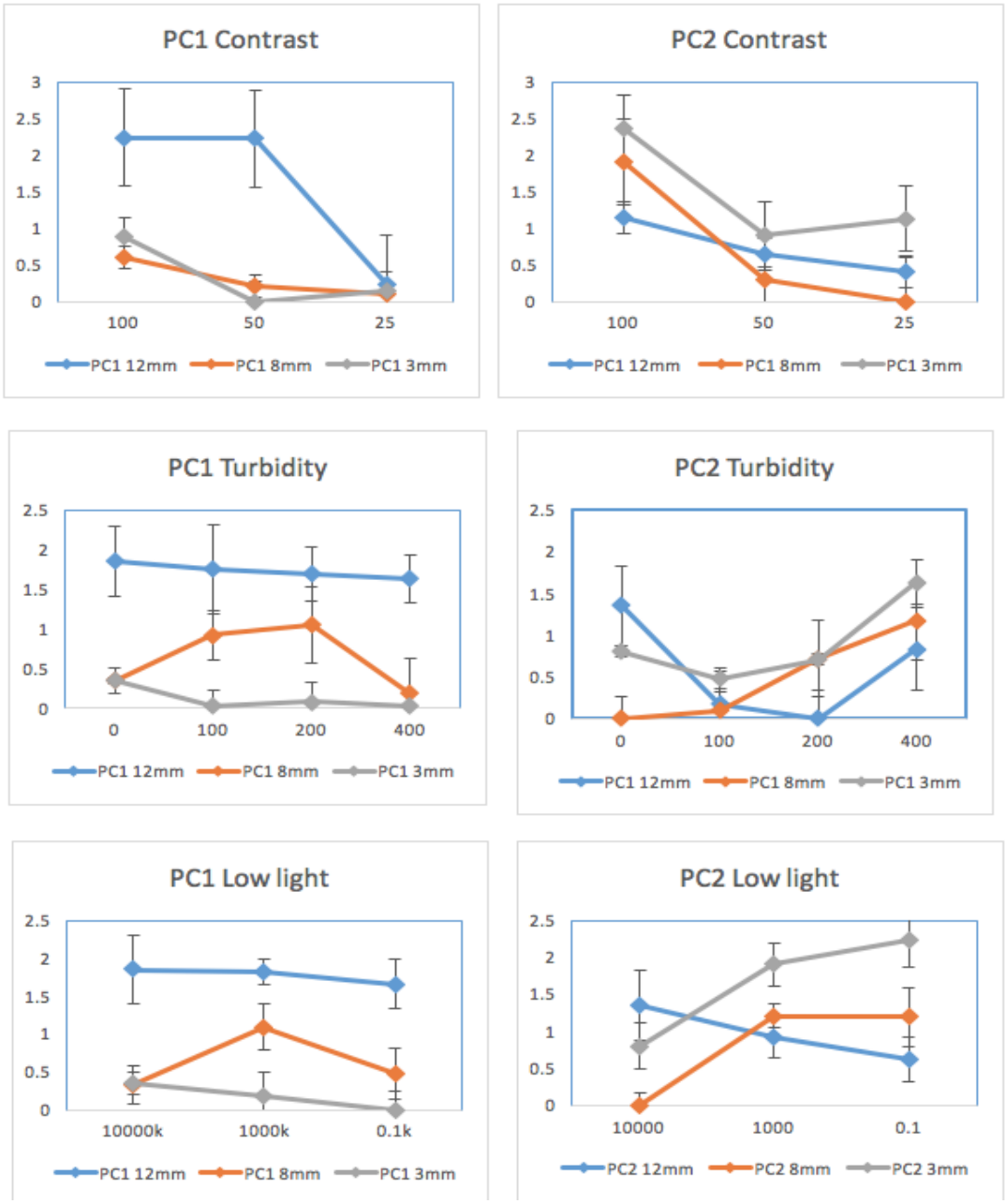


Figure 4.8: Comparison of PC1 (Disruptive) and PC2 (Mottle) responses to 3mm (fine), 8mm (medium) and 12mm (coarse) checks of varying contrast, turbidity and light-level. No significant difference is seen between PC1 values. There was no significant difference between turbid and low light PC2 however they were significantly different ($p<0.05$) to both lowered contrast

4.4 Discussion

4.4.1 Camouflage in relation to predator detection

Turbid and low light conditions affected camouflage differently than that on reduced contrast backgrounds. In the turbidity experiment, disruptive mottle Principal Component 2 pattern increases with increasing turbidity. At high turbidity in the natural environment visual predator prey interactions would occur at a shorter distance (Abrahams and Kattenfeld 1997). One can speculate from our findings here that the cuttlefish benefit from reducing contrast and especially disruptive patterning, resulting in an increase in mottle. In the case of these experiments the cuttlefish were still on a non-turbid background in the inner arena. To change to a completely mottled or uniform camouflage may be a disadvantage as a greater contrast between the background and the cuttlefish would arise and therefore draw attention of the predator. At some point, there would be an energetic trade off as suggested by Allen *et al* (2010) between the intensity of the camouflage and concealment from the predator This is possibly seen by the very subtle changes in the first two treatments of the turbidity concentrations where there was still some visibility of the squares in the outer arena. In respect to the cuttlefish detection by a predator Cartron *et al* (2013) suggest that cuttlefish polarisation vision would provide a reliable method to detect predator and prey in such turbid conditions. The possibility of the cuttlefish escaping either detection or capture may depend on this polarised vision ability along with the searching and reactive distances of the predator. In freshwater environments fish such as bluegills feeding reduces in turbid environments (Gardner 1981). As cuttlefish potentially also hunt in these conditions further studies of motion camouflage in turbid conditions would aid our understanding of more natural situations.

Both turbidity and low light environments provide cuttlefish with reduced light information but by different methods, however we can

see that the two experiments showed similar camouflage strategies. In the turbid environment, a mottled disruptive increased whereas the disruptive camouflage decreased. In reduced lighting camouflage was similar, but with an increase of white patches and papillae. As cuttlefish are active both day and night their ability to camouflage in reduced light conditions is not surprising. In fact, Allen *et al* (2010) found cuttlefish camouflage themselves in low light conditions comparable to nighttime levels. As numerous fish have adapted to low light conditions it was suggest the continued camouflage was for predator avoidance and possibly hunting success. As suggested by Allen *et al* (2010) producing camouflage has an energy expenditure trade off and if it was not necessary then could be costly to the organism. The similar camouflage strategies between the turbid and low light conditions should be considered due to the type of visual information presented to the cuttlefish.

4.4.2 Visual information to the eye of the cuttlefish

In nature light levels vary with the day night cycle. Decreasing light levels, and hence the rate of photon absorption, generally reduce visual information (Land and Nilsson 2012). What is interesting is the similarities in the responses to low light and the turbid environment where image quality is degraded as light is scattered by suspended particles (Johnsen 2012). However, when we consider the information that is being presented to the eye of the cuttlefish, both result in a smaller difference between signals received from surfaces with a given difference in reflectance, though of course the visual contrast (ratios of light reflected from different surfaces) is reduced by turbidity but not by varying light levels. The question remains as to whether camouflage that is displayed depends simply on what the cuttlefish can sense about the background, or rather the background is sensed correctly and then is adjusted to be optimal for defence against visual predators. In this context, it is notable that cuttlefish routinely enhance their contrast in camouflage when a predator approaches (Langridge

et al 2007; Langridge 2009), suggesting they do not simply make a general best match to the background, and may indeed take a predator's eye view of their camouflage.

If it were the case that camouflage was directly dependent on the optical image on hitting the retina, one could suggest a similarity to that of low contrast (Ultne-Palm 2002). However, we can see in the small and medium checks that PC2 reaction are significantly different from that of the lower contrast (Figure 4.9). The increase of papillae as well as the mottle effect although seen in a reduced contrast was not as prominent when the cuttlefish was exposed to turbidity or low light. This suggests it is not just the reduction of light level information hitting the retina that results in the displayed camouflage.

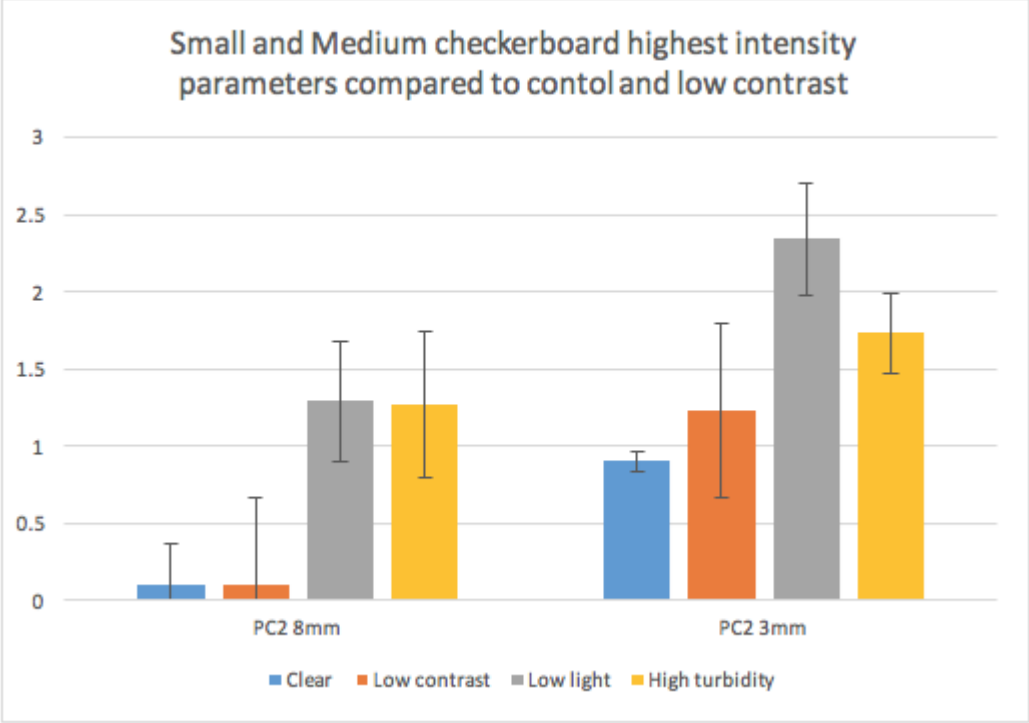


Figure 4.9: Comparison between full light and low contrast conditions and the highest turbidity value (0.2 a.u) and lowest light level (0.1 lux).

4.4.3 Role of polarisation

It may be that polarisation sensitivity enhances vision in turbid conditions (Schechner *et al* 2003; Cartron *et al* 2013). Although the use of polarisation information was not tested in these experiments it may well be relevant to cuttlefish camouflage, especially in turbid water. Cartron *et al* (2013) and Pignatelli *et al*(2011) both showed the presence of a predator in turbid water caused cuttlefish to react in an anti-predator response. However, neither of these described in detail the camouflage of the cuttlefish before the presence of a predator, so it remains unclear how much cuttlefish use polarised vision in a turbid environment for camouflage versus predator detection. Cartron *et al* (2013) suggests the polarisation channels are used for predator and prey detection in low contrast situations such as when luminance contrast is low. It is therefore possible that polarised vision is not used for camouflage decisions but more in response to predator and prey detection.

4.5 Conclusion

Reaction to turbid and low light levels show similar responses in camouflage, suggesting similar mechanisms are employed when there is reduced light and high scatter information. Cuttlefish continue to camouflage under these conditions, however, with reduced visual information cuttlefish increase mottle patterning and papillae. It is suggested this is to break up their overall outline to predators as high contrast patterns would have greater detection by predators. Further studies need to be conducted to determine what role if any polarised light has in these conditions to aid camouflage.

Chapter 5 : Shadow perception

5.1 Introduction

The optical image on the retina – a pattern of varying light intensity – needs to be interpreted by perceptual mechanisms in the brain to infer its physical cause, and hence to allow the animal to respond appropriately to those causes. A simple example of the problems that need to be solved by the brain is the distinction between the effects of varying illumination – shadows and highlights – and the effects of changing material properties in the reflective surface. For a cuttlefish that wants to camouflage itself in shallow water this is a potential problem, because shadows cast from above will of course be projected onto the animal so there is no need for it to match them. This chapter investigates using simple checkerboards to see whether cuttlefish can discriminate cast shadows from above to that of a patterned surface. This to our knowledge has not been investigated in cuttlefish before.

5.1.1 Light properties in a water medium

Organisms use various different cues of this visual information to perceive the world around them. Edge detection, area, visual depth and contrast all feed into the way the visual world around them is processed. To what degree the information is processed is dependent on the medium of which it is transmitted and the ability to gain the visual information in such medium. In water, certain visual cues may be lost or affected because of environmental factors such as turbidity, obstructions or light levels. As we have seen in previous chapters when the visual information is altered, cuttlefish camouflage responds in varying degrees. When the light is blocked entering the water column the result is a cast shadow. Shadows are used by animals to

place objects in context but also gives information about objects in a 3-Dimensional space (Rosenthal 2007). In the aquatic environment as the light source is often from above objects such as rock outcrops or even other organisms cast shadows onto the seafloor, as do ripples at the water surface. To what effect the shadow has on camouflage decisions has yet to be investigated in cuttlefish. This experiment is a preliminary study to determine if there is discrimination between the shadow and artificial background.

5.1.2 Cuttlefish environmental cues

Kelman and others (2007) found evidence that cuttlefish can sense the difference between 2D photographs of a gravel substrate and real 3D gravel, even when both are placed under a layer of Perspex. Other studies in this Thesis and elsewhere (Barbosa 2008, Ulmer 2013) have shown cuttlefish can recognise objects such as stones, plants and vertical structures. The fact the cuttlefish can distinguish the objects suggest they have the ability to detect and utilize the visual cues in their environment to recognise objects and their 3-D form. These objects would all give edge information through the form of shadows, but also reflectance of the light of the object. Using simpler stimuli many studies (e.g. Zylinski *et al* 2009) have found cuttlefish can sense 2D patterns such as a checkerboard pattern where the information is from reflected light rather than changing illumination (that is shadowing). In this experiment we will investigate, using camouflage patterns, how shadows in the form of a projected image versus reflected information are perceived by cuttlefish. The decision was to use a projected image over that of a backlit object. The reason was to try and reduce to the influence of having an object directly above the cuttlefish.

The aims of Chapter 5 are:

1. Can cuttlefish differentiate between low contrast, projected image and reflectance
2. How do cuttlefish respond to a projected image

5.2 Method

The main purpose of this study was to compare responses to high contrast project patterns (i.e. shadows) to similar printed checkerboards. To control for the minor effects of distortion in the water column and to distinguish quantitative from qualitative effects (did the cuttlefish see the shadow pattern as a low contrast reflected pattern or as something quite different). We used 100%, 50% and 25% contrast checkerboard with 120mm checks, and in addition (50% Gaussian) blurred checkerboard. A uniform grey background was used as a control (Figure 5.2). Checkerboards were projected into the filming tank with a 3M mpro-150 projector which was mounted on the filming window of the tank (Figure 5.1). Once the cuttlefish were settled photos were taken every minute for 5 minutes. One photo was then randomly selected for each cuttlefish to be analysed.

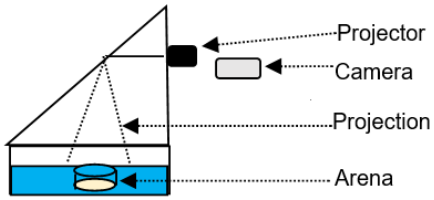


Figure 5.1: Set up of experimental tank. Projector light was bounced off a mirror into the filming tank

Size(cm)	Michelson contrast (a.u)						Gaussian Blur	Grey
Contrast of Black Square	100		50		25		100	
	B	P	B	P	B	P		
Large (1.2)	0.9	0.85	0.44	0.32	0.2	0.22	0.8	0

Figure 5.2: Table divided into treatments showing level of contrast in each size and treatment. B=Printed Background, P= Projected Background

5.3 Results

KMO score 0.751 explains 47% first 3 PC's

Principal Component 1(PC1) has more disruptive camouflage patterning with Principal Component 2 (PC2) and Principal Component 3 (PC3) being a more mottled effect (Figure 5.3).

Cuttlefish seem to differentiate between the projected image and that of reflected or low contrast, shown by a significant difference between the background and projected image in PC1 and PC3 ($P<0.01$). Large checks followed a reduction in PC1 but retained a mottle camouflage at low contrast.

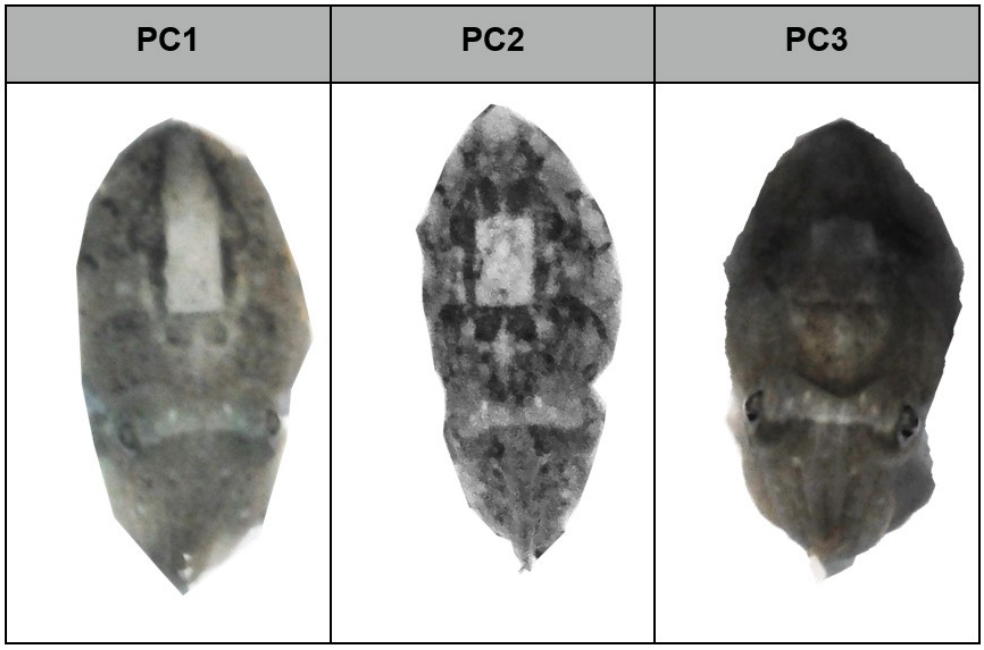


Figure 5.3: **First three Principal Components**

Principal Component	PC1	PC2	PC3
White Posterior Triangle			
Median Mantle Stripe			
White Square			
White Head Bar			
Paired Mantle Spots(Anterior)			
White Splotches			
Mantle Margin Scalloping			
Paired Mantle Spots (posterior)			
Visible Cuttlebone			
White Landmark Spots (White Square)			
White Major Lateral Papillae			
White Landmark Spots (Head)			
White Neck Spot			

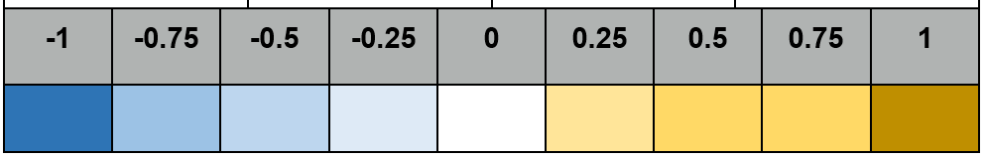


Figure 5.4: Principal Component matrix for projected, low contrast and blurred treatments.

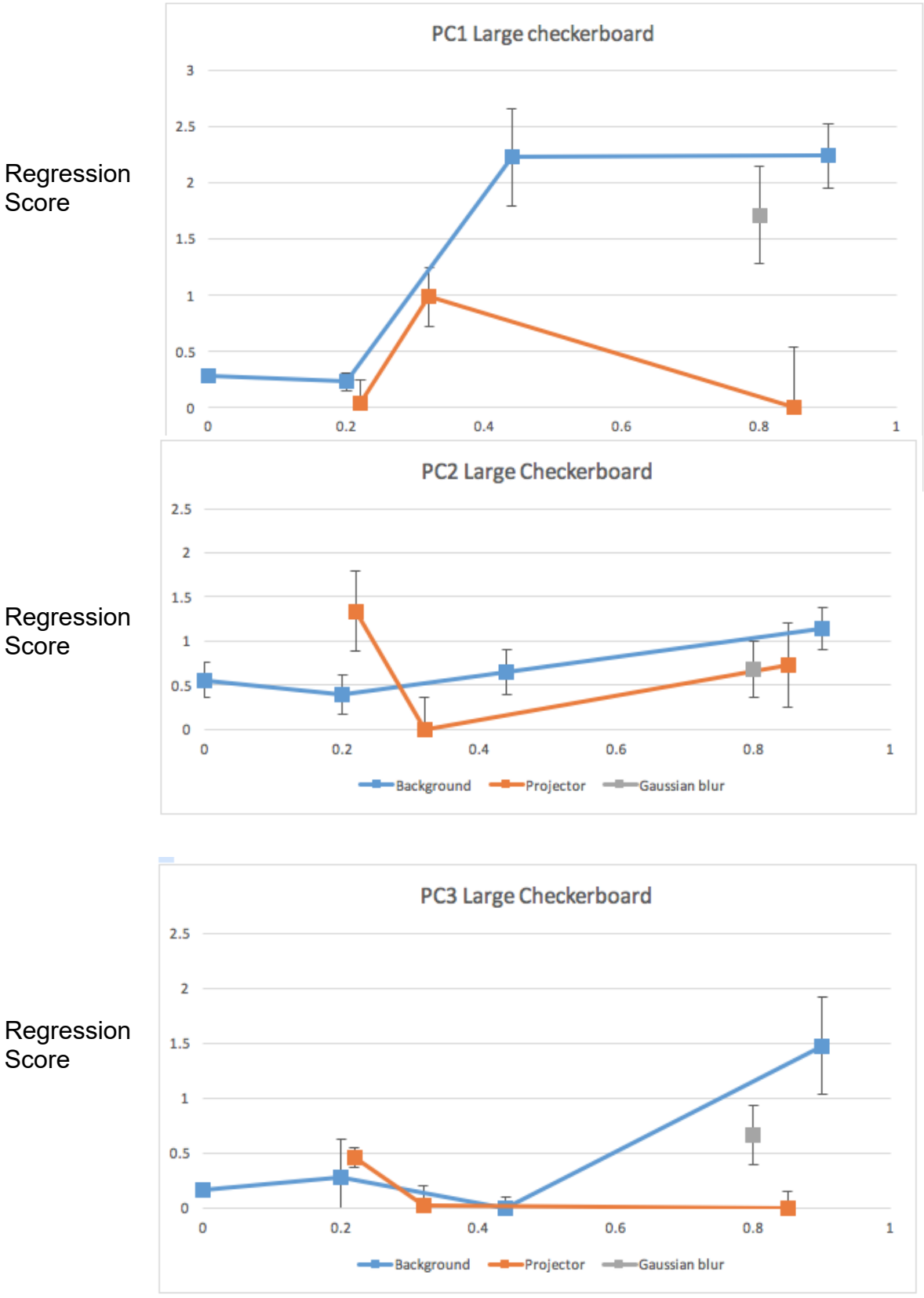


Figure 5.5: Graphs of projected image versus checkerboard contrast divided into to the 3 Principal components. Blurred and grey treatments are also plotted to compare against projected and background images.

For PCs 1 and 3 responses to the 25% contrast projected and printed backgrounds were in most cases very similar to one another and to the uniform grey ($p>0.05$) (Figure 5.5). If we compare these to Zylinski *et al* (2009) we can see that in the large check as contrast decreases, white square was reduced which is similar in PC1 and PC3.

5.4 Discussion

We have found here that the response of the cuttlefish to high contrast shadows is close to a uniform background. That is the physical surface on which the animal is resting, compared to a visually similar printed checkerboard, to a 50% contrast checkerboard or the blurred version. The responses to the 25% contrast checkerboard, the projected pattern and the uniform surface are not separable. Thus, it is reasonable to conclude that the animal 'sees' shadowed surface either as being physically uniform in reflectance – as it is – or possibly a low contrast pattern. The most variation is seen in PC1 and PC2. PC2 increased as checkerboard contrast was reduced, whereas the projected checkerboard decreased as contrast was reduced. The results are all the more striking given the rather artificial nature of the projected checkerboard, compared to shadowing caused by the surface ripples or seaweed in nature. We can only speculate how the cuttlefish is able to make the distinction between illumination and reflectance. It might perhaps see the pattern beneath its mantle when it casting its own shadow.

In the wild, shadows would give important information for depth perception and for spatial information. In this study the shadows were cast from above rather than a directional light sideways. Therefore, the shadows were cast down onto the cuttlefish as if an object was above rather than from the side. Further studies would be need to be

conducted to determine how directional light alters camouflage. In the more natural situation it would be conceivable the object casting the shadow would also influence a possible cuttlefish camouflage decision. When we look at the cuttlefish in this experiment we can see they still respond to the cast checkerboard pattern, suggesting they still use edge detection in camouflaging. A white square expression would be a good indication of this. It is possible the cuttlefish actually use the shadow that is being cast upon them as a form of camouflage and therefore a greater chromatic response would not be required. As this was a preliminary study with a new experimental design, more investigation would be needed to expand on this possible hypothesis.

5.5 Conclusion

Overall, cuttlefish seem to be able to differentiate between a projected and reflected image. Cuttlefish appear to treat projected images similar to a lower contrast. The implication and scope in different settings would still need to be examined before being able to draw overriding conclusions.

Chapter 6: Motion Camouflage

6.1 Introduction

Predators drive the evolution of animal defences, which include morphology, behavioural adaptation, warning signals to conspecifics and determination of the predator's location (Caro 2005). The ability of predators to recognise prey is vital, and recognition depends first on seeing the prey as an object distinct from the background. Here, several environmental factors need to be taken into consideration, notably the light source, the background including its spatial and temporal heterogeneity and light transmission through the medium, especially in water (Rosenthal 2007). Together these factors mean the effectiveness of a given type of cryptic camouflage will be dependent on the visual environment, as well as the distance of the viewer and their visual abilities (Ruxton *et al* 2004).

6.1.1 Motion Camouflage

Crypsis, based on background matching, is likely to be most effective when an animal is stationary. Movement causes two problems. Firstly, the new location may offer a different background, but more importantly that movement itself breaks camouflage by allowing the predator to recognise a coherently moving object. There is evidence certain camouflage strategies work by confusing predators when prey is moving. Notably so-called 'dazzle' patterns whereby markings such as high-contrast bands, stripes and zig zags might reduce the predator's judgement of speed and trajectory (Stevens *et al* 2011), but dazzle patterns are likely to be conspicuous when the prey is stationary. Of course, an animal such as the cuttlefish that can change its camouflage may not need to compromise between motion and stationary camouflage, by selecting the appropriate pattern for each condition. This offers an opportunity to study what techniques may be employed to reduce detection while in motion

6.1.2 Cuttlefish motion camouflage

While there are several studies on camouflage of stationary animals (Hanlon 2007, Chiao *et al* 2010), motion studies have focused on determining camouflage on standardised backgrounds without predatory influences (Zylinski *et al* 2009b, Josef *et al* 2015, Josef *et al* 2016). Contrary to some predictions (Stevens *et al* 2011), Zylinski *et al* (2009b) found cuttlefish did not use high contrast motion dazzle, instead the contrast of body pattern components was reduced during movement. Josef *et al* (2015) went one step further describing colour matching through movement. However, it was not determined how this translates to an environment where other factors such as hunting may be relevant. Casual observation shows cuttlefish often change coloration when feeding. Messenger (1968) and Adamo *et al* (2006) documented postural and chromatophore change in cuttlefish camouflage when feeding, but focused more on posture and behavioural aspects of the prey capture. Messenger (1968) identified three stages of prey capture: attention, positioning and seizure. In the attention stage the cuttlefish responds to the prey item by either turning its head or by colour change. During positioning the cuttlefish either retreats or approaches along the prey-body axis, this is when the cuttlefish is directly facing the prey. Finally, the cuttlefish seizes the prey. During these movements cuttlefish often undergo substantial changes in colouration, whose function is unclear. Are they aggressive, or defensive?

This chapter investigates these changes, and examines how prey influence motion camouflage of cuttlefish both in uniform and in varied environments. Just as responses to predators depend on the level of threat and the type of predator (Langridge *et al* 2007; Langridge 2009), the feeding cuttlefish seem to deploy their repertoire of coloration patterns in a complex and flexible manner. The choice of coloration pattern depends on a number of factors

including the background, the type of prey and the particular movements used during an attack.

The aims of Chapter 6 are:

1. To determine the behaviour of prey capture over distance and substrate
2. To describe and analyse the different camouflage expression during predation
3. To investigate how prey items effect camouflage

6.2 Method

All experiments were conducted at Brighton Sea Life centre in a designated filming tank. The 10 animals ranged from 120-360 days in age with 70mm-90mm mantle length. Backgrounds were either sand or small (<15mm diameter) pebbles. The cuttlefish were presented with two food types, shrimp (*Crangon crangon)* or crab (*Carcinus maenas)* with approximate total length of 30mm in Shrimp and 27mm carapace in crabs. We recorded distance to the prey item, the direction of movement of the cuttlefish and the predation behaviour as prey were more or less stationary after introduction. Video was taken of the prey interaction and stills were taken for the three stages described by Messenger (1968) (Figure 6.1), but in addition to attention and orientation pre attack and post attack (seizure) were separated This was repeated for all 10 cuttlefish over the varying distances, on two backgrounds stones and sand and for both types of prey.

Attention	***Positioning***	***Seizure***
		

Figure 6.1 The three stages of prey capture described by Messenger (1968)

Cuttlefish images were scored blind and graded by eye with the pattern components scored on a three point scale as according to standard procedure (Refer to Chapter 2). Scores were entered into a SPSS v22 spreadsheet. A principal component analysis was then conducted on the grading scores and subsequent component regressions analysed by ANOVA. The first four components (PC's) explain for 40% variance with a KMO score of 0.681. PC1 has a

darkened head region with some disruptive characteristics such as a white square and median stripes. PC2 has light disruptive characteristics. PC3 has a darkened head region with certain other dark components. PC4 is more uniform with some stippling.

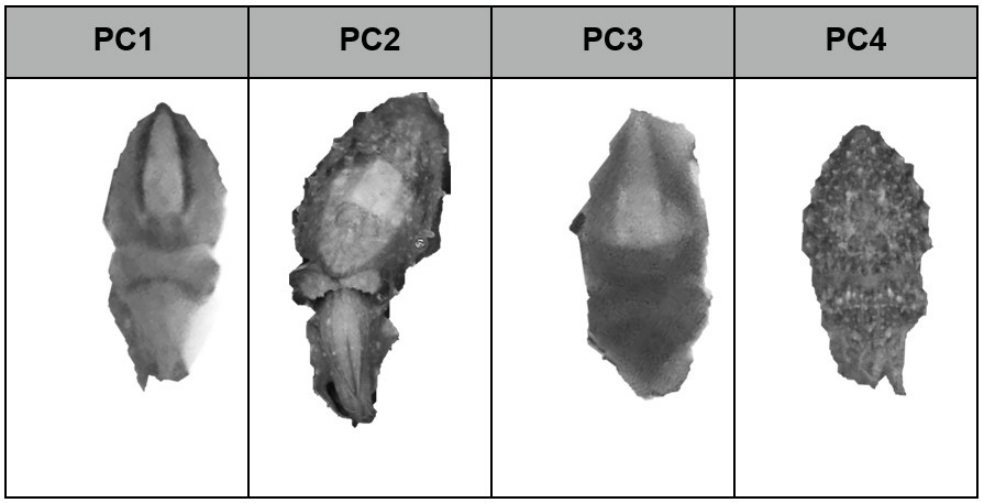


Figure 6.2: Table of first 4 principal components

Principal Component	PC1	PC2	PC3	PC4
White Square				
White Posterior Triangle				
Median Mantle Stripe				
Anterior Head Bar				
Paired Mantle Spots Posterior				
Posterior Head Bar				
Posterior Transverse Mantle Bar				
White Mantle Bar				
Anterior Transverse Mantle Bar				
White Head Bar				
Anterior Mantle Bar				
Posterior Mantle Bar				
White Landmark Spots (Arms)				
Mantle Margin Scalloping				
Paired Mantle Spots Posterior				
White Splotch				
White Major Lateral Papillae				

-1	-0.75	-0.5	-0.25	0	0.25	0.5	0.75	1

Figure 6.3: Principal component matrix for motion camouflage with values greater than 0.3 values. KMO score was 0.681 and 40% of the variance was explained in the first four factors

6.3 Results

6.3.1 Movement Response

Behavioural responses to prey items were varied and could be split into instances of orientation movements without translation (henceforth ‘stationary’), movement across half the arena and movement across the whole arena.

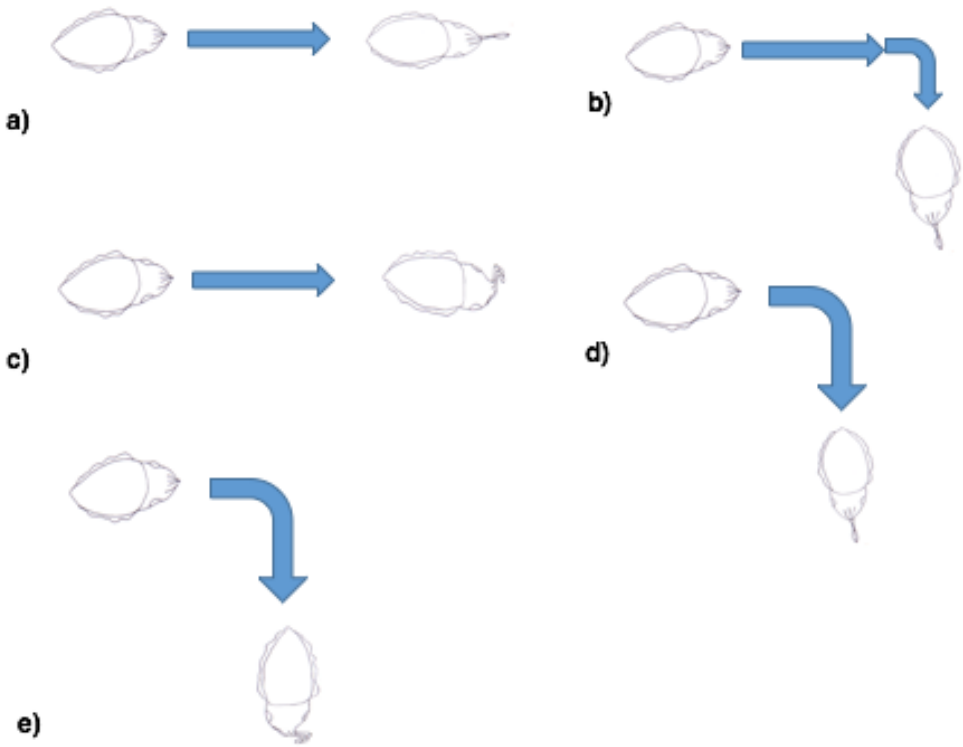


Figure 6.4 : Diagram of different behaviour exhibited on approach to prey item a) straight attack b) Straight attack with curved c) Straight attack with arm raise d) curved attack e) curved with arm raise

Cuttlefish movements could be broken down into further categories (Figure 6.4). The capture method was dependent on distance and prey (Figure 6.5). At shorter distances a curve with an arm wave was used 57% of the time when a crab was the prey item (*n=14*), this reduced to 36% when the distance was across the arena (> 2 mantle lengths) (P<0.00) A straight path across the arena with an arm wave was the next most observed within the crab treatment at 21% (*n=19*). When shrimp was prey, a straight attack was seen 50% of the time when halfway across the arena (*n=20*), this fell to 30% when it was a longer distance across the arena. In contrast a curved attack with arm wave increased to 30% across the arena from 15% half across the arena. This suggests different techniques are needed over longer distances.

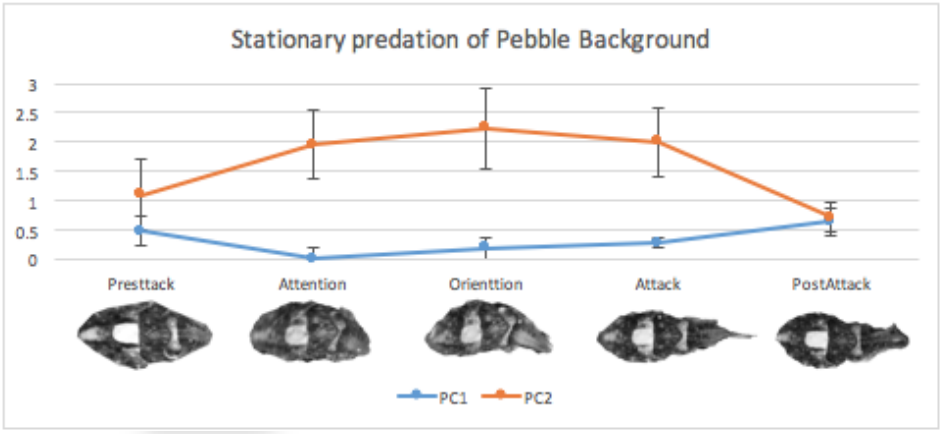
Prey Type	Movement	Percentage behaviour	
		Half across arena	Across the arena
Crab		(*n=14*)	(*n=19*)
	Straight	14	16
	Straight with curve at end	7	10
	Straight with arm wave	0	21
	Curved	21	16
	curved with arm wave	57	37
Shrimp		(*n=20*)	(*n=13*)
	Straight	50	31
	Straight with curve at end	15	23
	Straight with arm wave	5	8
	Curved	15	8
	curved with arm wave	15	31

Figure 6.5: Cuttlefish behaviour broken down into food type and distance. Distance effects the behaviour used (p<0.00) . Across the arena has more variability in behaviours used than that of half across the arena.

6.3.2 Camouflage in motion on different backgrounds

Strength of the Principal Components' expression was dependent on distance and background. On a pebble background PC2 expression differs significantly (p=.0.004) between stationary predation (Figure 6.6a) and when there was movement across the arena (Figure 6.6b). In stationary predation PC2 increased during the orientation and attack stages. With movement across the arena PC2 decreased during the orientation stage, but peaked at the attack stage. PC1 also showed a significant increase (p=0.00) during the attack stage on a pebbled background (Figure 6.7).

a)



b)

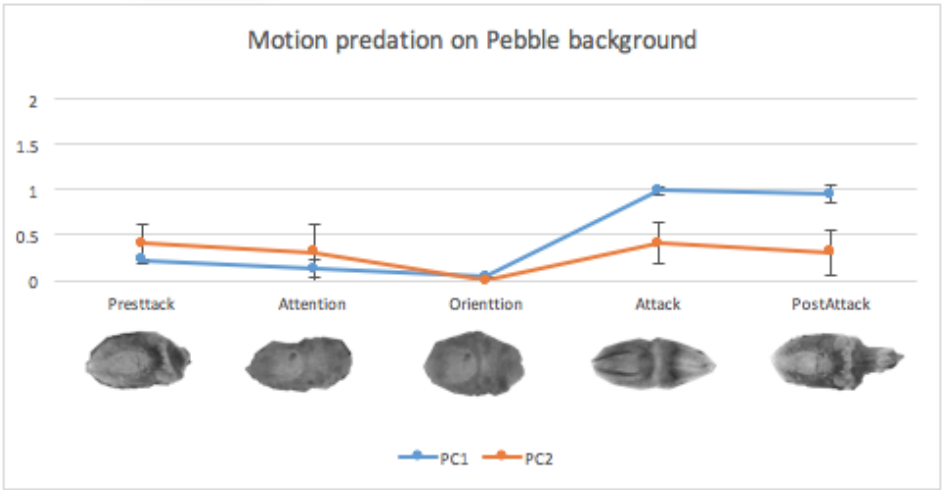
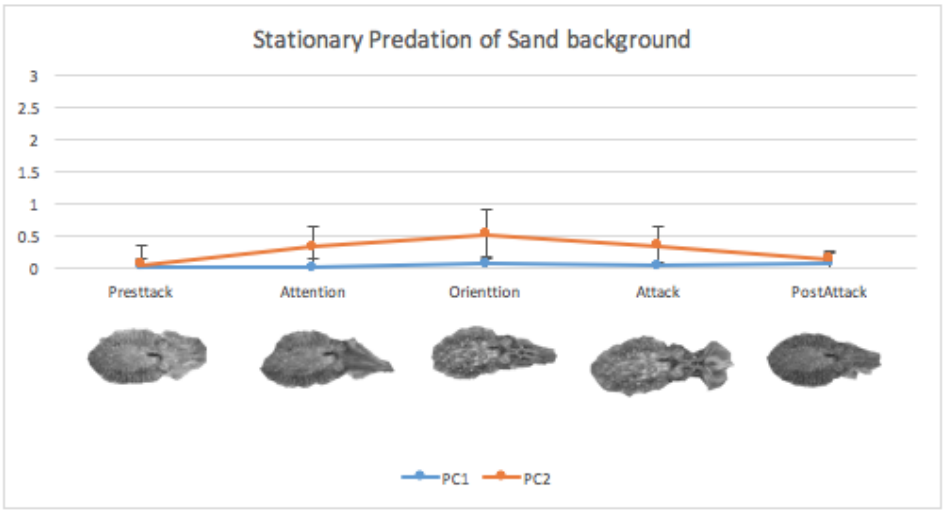


Figure 6.6 a) Stationary predation camouflage on pebble background where PC2 increases over attack stages b) Motion predation camouflage where PC2 shows only a slight increase at attack stage

On a sand background PC fluctuations were reduced in the stationary predation. PC2 increased slightly around the orientation but was not significantly different from PC2 in motion the predation. Whereas PC1 showed a gradual increase through the different stages of the motion predation with a non-significant ($p>0.05$) drop off in post attack



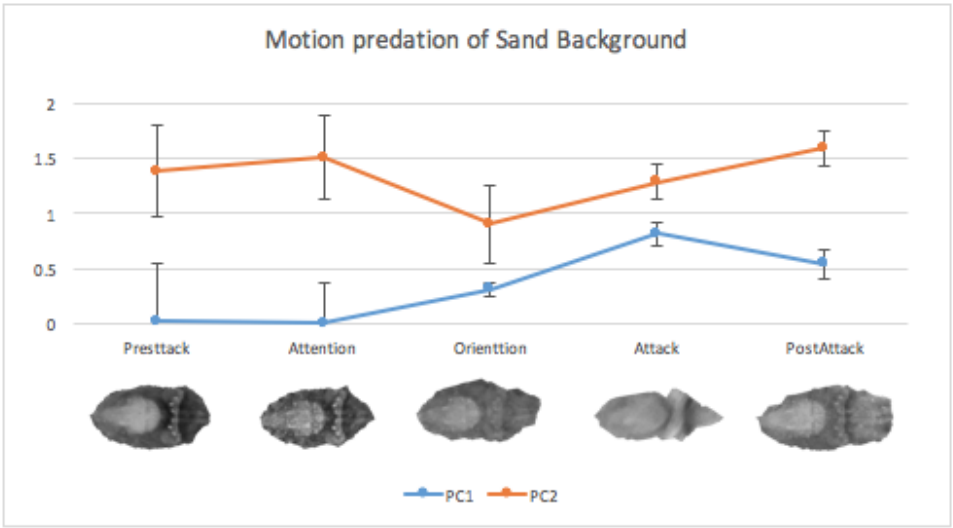


Figure 6.7: a) Stationary predation camouflage on sand background where PC2 increases during orientation but not significantly b) Motion predation camouflage where PC1 increases over orientation and attack stage.

6.3.3 Camouflage in motion compared to prey type

When food type and distance are analysed we can see there are similar trends in both PC1 and PC2 when travelling across the arena ($p<0.05$) (Figure 6.8) When motion was only half way across the arena, PC2 had a greater fluctuation at the orientation stage when faced with crab as prey.

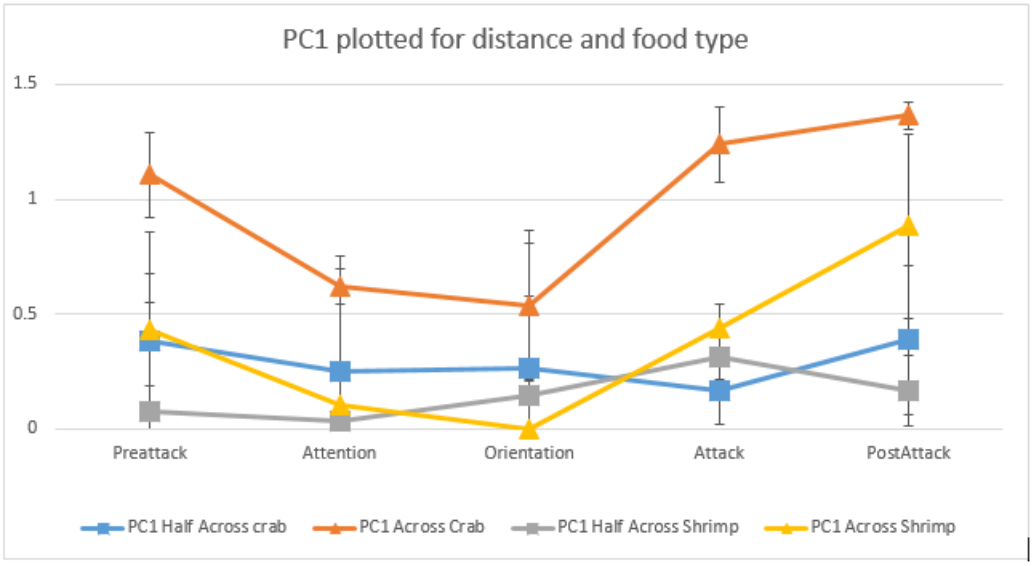


Figure 6.8: PC1 have been divided into distance and food type. PC1 increases at the attack stages over distance in both prey types

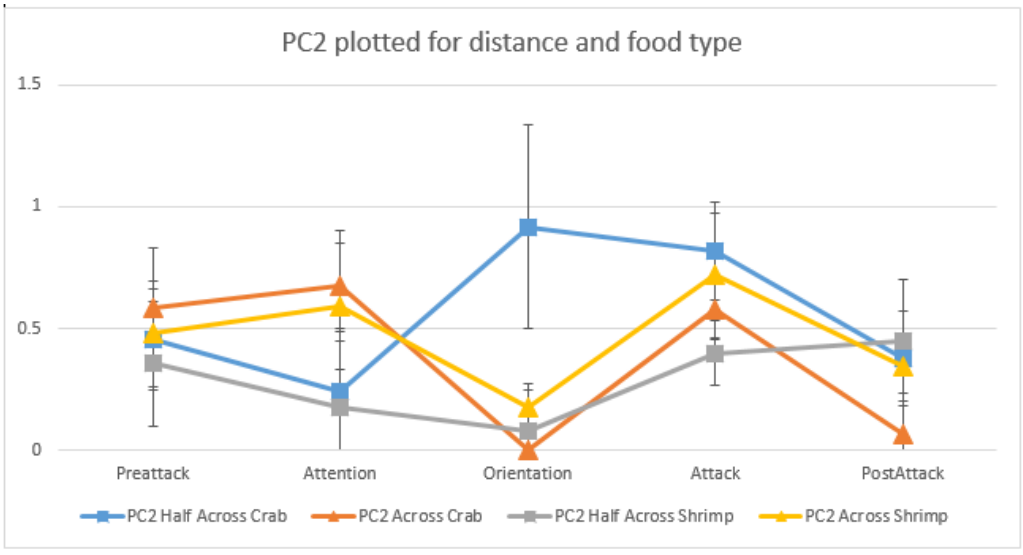


Figure 6.9: PC2 divided into distance and food type. PC2 decreases at orientation stage in both types of prey with the exception of crab half across the arena

6.4 Discussion

6.4.1 Behaviour in response to prey

Both Boal *et al* (2000) and Adamo *et al* (2006) found cuttlefish avoided the claws when attacking crabs, while Adamo *et al* (2006) found they were seized in different ways: fish and shrimp with the tentacles, but an arm grab for crabs. Similarly, we found different approach movements to prey. For crab a curved attack with arm wave most frequent in both half arena and full arena movement. In comparison, the shrimp attack was more varied, from short distance there was a straight attack on 50% of occasions. When the distance increased to the width of the area straight attacks on shrimps decreased to around a third of the total. These behavioural differences could be due to the defensive capabilities of the prey. Crabs can injure a cuttlefish, so distraction of the prey and attacking from the side or back would reduce risk of a nip. In contrast the shrimps *(C. crangon*) used in this study had no such defences, but are more agile. At a short distance a straight approach is quick as the cuttlefish can use their tentacles for the attack. At a longer distance the chance of being spotted by the prey item is greater. Srinivsan and Davey (1995) point out a curved path can allow the cuttlefish to appear as stationary object if it keeps its initial distance to the cuttlefish being in the constraint line of the prey's vision. If we compare the cases where cuttlefish are moving as are the prey, we can see some similarities with Srinivsan and Davey (1995) predicted trajectories, but further study would be needed to confirm if the cuttlefish are indeed using their motion camouflage strategy (Figure 6.9)

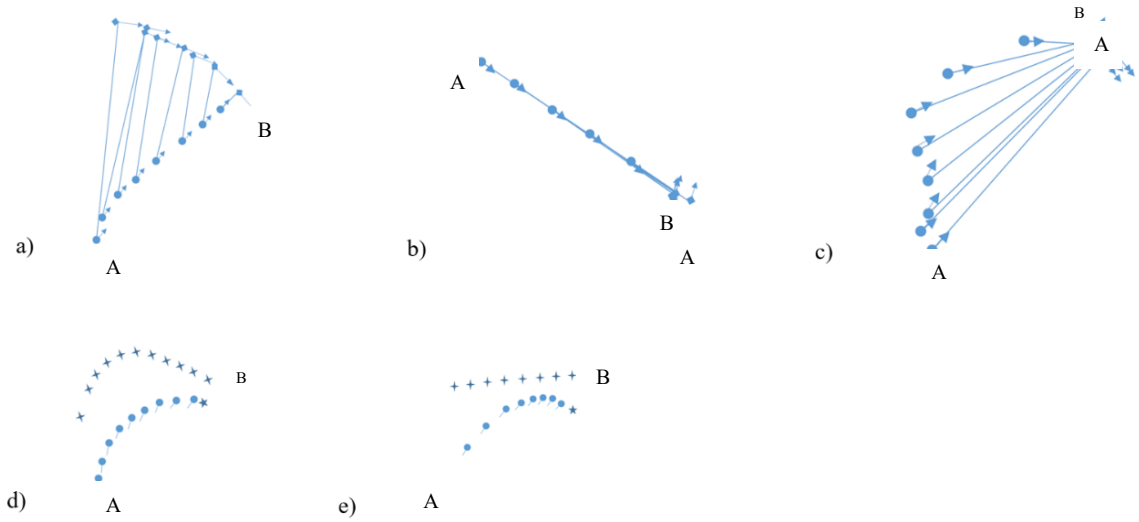


Figure 6.9: Trajectory of cuttlefish and prey with Srinivsan and Davey (1995) algorithm for comparison. Diamond and star designates prey and circle predator. Body orientation is designated by directional line a) Curved predating cuttlefish with moving shrimp b) Straight predation cuttlefish with stationary prey c) Curved predating cuttlefish with stationary prey d) Prey and predator moving at varying speed to a moving target e) Constant moving prey in straight line to a fixed target with predator moving.

6.4.2 Distance in relation to prey

For both types of prey when distance was increased so did the frequency of arm waving behaviour in both straight and curved attacks (Figure 6.5). Cuttlefish raise their arms in response to visual stimuli, probably for camouflage amongst 3D objects (Barbosa *et al* 2012), but here the fact that arm wave overall increased with distance for both the straight and curved attacks (Figure 6.5), is consistent with the logical possibility that cuttlefish need to confuse prey when attacking from a long range. Hanlon and Messenger (1988) suggest the arm wave 'mesmerizes' the prey, and its use could be due to individual preference from early learning, or is perhaps genetic. However, it could also be a distraction technique, or possibly to mimic a prey item for fish. It is noteworthy that crabs living in complex visual environments (such as rocky shores) have a pronounced acute zone in their visual field (Nalbach *et al* 1989), and movement around the equator of the visual field can produce eye

movements. As such there is a fixation response with the fixation on the object beginning when something is approximately 28 degrees above the horizon. As the eye moves the input images need to be translated and integrated into the visual system. An image close to the crab in the vertical plane would have to distinguish distance, which could be counterbalanced by image motion in each eye. If the cuttlefish is able to conceal the rest of its movement, then waving of the arms could indeed 'mesmerize' the prey and confuse motion information. Oliva *et al* (2007) showed that depending on direction and colour of the stimuli different neural and behavioural responses are elicited. Darker approaching objects showed the greatest response of evasion, however a lateral moving object or light object showed a reduction in neural response and evasion. It is interesting to note arm waving coincides more with a curved approach with both shrimp and crab prey items.

6.4.3 Background influence on motion camouflage

As the cuttlefish moves it is important to also take into consideration their visibility to predators as well as prey. There are strategies that a cuttlefish may implement to reduce their visibility to the predator. Langridge *et al* (2007) suggested several things needed to be taken into consideration when considering predatory threat to a cuttlefish. The type of predator, the proximity of the predator, direction of movement and substrate. It was found that behavioural responses were strongly associated with the overall threat level during encounters with threatening species. Staudinger *et al* 2013 supported this idea that different predator elicited different responses when cuttlefish were exposed to flounder, seabass and bluefish. It was also suggested that the defences were highly context dependent. In active searching predators such as bluefish cryptic behaviours may be more important. However, if the cuttlefish moves a couple of strategies could be used. One would be to use motion dazzle, which would confuse the viewer of the direction and speed of

the cuttlefish as it moves (Stevens *et al* 2008). Another would be to reduce contrast to confuse speed information or another pattern to reduce detection. (Zylinski *et al* 2009b). A dazzle display consists of high contrast markings to inhibit the predator's judgement of speed for example in snakes, fish and mammals. (Stevens *et al* 2011). Capture rates of such displays have been compared with that of low contrast and found uniform grey targets were missed more often than a patterned target (Stevens *et al* 2011).

Zylinski *et al* (2009b) found cuttlefish reduced high contrast characteristics when moving over substrate, suggesting it was to reduce detection by predators. Josef *et al* 2015 went one step further suggesting that cuttlefish showed background matching during motion. However, there was no perceived threat or motivational stimulus in these studies. In comparison, this study shows differing distance produces differences in camouflage technique. On a pebble background a stationary response causes some lightening of chromatic features (PC2), whereas movement background causes a marked increase in PC1 at the attack stage. On sand backgrounds the pattern of change is similar, but weaker than on pebbles ($p>0.05$). PC2 increased, but not significantly, during the orientation in stationary attacks, and there PC1 increased during the final phases of the attack when the animal needed to move to capture its prey. It could be suggested that the lower contrast patterns on sand are a result of the animals need to maintain camouflage on a conspicuous background during the moving stage of prey capture.

6.5 Conclusion

Overall both the prey type and its range affected coloration. At a greater distance PC1 increased around the attack stage in both shrimp and crab. The importance behaviour has on the stimuli and strength for the change in colouration has yet to be determined.

However, due to the increase in distance it would be likely that there is a greater risk of detection by prey and predators. Therefore, different techniques may need to be used to counteract this. Perhaps a larger movement entails the use of motion camouflage.

Chapter 7: General Discussion

7.1 Objects in the environment

There are three ways in which behaviour can influence camouflage. Choosing a microhabitat to increase similarity to background, orientation alignment to the background to increase localised similarity or choosing a background to increase scene complexity (Stevens and Merilaita 2011). When proximity of objects was taken into account cuttlefish behaviourally chose whether to sit near an object based on its optical properties such as reflectance and size. In objects with less reflectance cuttlefish used them for possibly mimicry. In shrimps where Sargassium habitat is varied, camouflage and plant-part mimicry can depend on the size, shape and colour of the shrimp (Hacker 1991).

The size of these objects and overall location in the cuttlefish's vision played an important role in camouflage. Objects in a vertical plane had a significant impact on the expression of camouflage, but also the distance on the horizontal plane. Even at a greater distance, objects and backgrounds had some influence on the camouflage expression often resulting in a mixture of backgrounds in the camouflage. This outlines the complexity to which the cuttlefish take in their surroundings for camouflage. It may be that they may have a similar strategy to that described by Josef *et al* (2014) regarding octopus. It has already been determined by other researchers (Kelman 2007) that depth cues were an important factor. It was suggested by Zylinski (2009) that cuttlefish vision uses multiple cues in visual tasks with several parameters involved. With Josef *et al* (2014) not only the immediate surroundings were considered in camouflage decisions. The findings in Chapter 3 show similar

strategy in addition to a behavioural component. What drives the behavioural component is almost certainly the possibility of concealment. Skelhorn *et al* (2011) suggest that it is the predators cognitive strategies (such as recognition and identification) rather than sensory capabilities that are the selective force driving the evolution of masquerade. Cuttlefish predators such as sea bass have a high visual acuity and it is possible that this may be one of the driving factors to resembling stones on a uniform background where there would be little cover.

7.2 Reduced visual information

Camouflage displayed by cuttlefish varied significantly dependent on the visual input and the parameters of the surrounding environment (Figure 7.1). In an aquatic environment parameters can have high variability due to factors such as ocean current, sedimentation and light levels. When optical information is altered by these variances such as low light or high turbidity, cuttlefish adjust camouflage as demonstrated in Chapter 4. The level of expression of disruptive components is diminished with lighter components increasing, giving a more mottled appearance, possibly breaking up the overall outline of the components and the cuttlefish. This would aid in the reduced detection by a predator as in these conditions predators would have to be closer to detect a prey item. *Gobiusculus flavescens* show a significant reduction in reaction distance in both reduced illumination and increased turbidity (Utne 1996). In turn, distance of objects, and in this case predators, plays a major role in the expression of camouflage in cuttlefish. In fish predator studies Ranaker *et al* 2012 found that there was a reduction in striking distances in pike when only using visual cues and that the prey's escape distances increased in turbid water. However, when chemoreception was present there was no significant difference in strike distance in turbid

water. This suggests that predators that use multiple cues to hunt would not be as greatly affected when hunting cuttlefish in a turbid environment.

The level at which the cuttlefish can detect reduction of visual cues is still a question under investigation, but when looking at shadow and normal light conditions we can see cuttlefish can indeed detect a difference between reflectance versus luminance as demonstrated in Chapter 5, and that they treat shadows similar to a low contrast background of similar configuration rather than a uniform grey background or a blurred image. How cuttlefish use this in a more natural situation is still to be determined. It may be a shadow is used in camouflage due to possible concealment if the cuttlefish is uniform. The previously described chapters were testing environmental parameters that may be present in the natural environment. However, all the camouflage was in relation to stationary camouflage, where as in the ocean cuttlefish would be moving dependent on stimulus such as predatory pressure or hunting.

7.3 Motion and predation

By looking at situations where cuttlefish were stimulated to move over varying distances and backgrounds, we can see if components that are expressed in stationary situations are held or altered during movement. Zylinski (2009) suggested cuttlefish altered their camouflage dependent on movement by lighting contrast. This was conducted by a simple disturbance. Josef *et al* 2015 took it one step further and described a sigmoidal, colour-changing mechanism, which they suggested reduced detection. In this experiment, I wanted to test if, when there was motivation such as food, whether these observations held true or were altered due to the presence of the prey item. In Chapter 6 it was shown that although they do alter the

camouflage when moving, it is not as straight forward as just reducing contrast of the components when moving towards a prey item. Not only did behaviour change depending on distance and prey item, but also camouflage changed over different backgrounds. The approach to prey seemed to reflect the possible risk of detection or possible damage in the case of the crab. The motion camouflage displayed in some cases did resemble techniques to confuse motion detection. It appears the head region has separate expression than the mantle. It is suggested here that this is due to predator versus prey viewpoint. Mantle expression would be to confuse potential predators who may be viewing from above in comparison to the prey item, which would see the head region coming towards it. If both regions showed similar camouflage the effectiveness may be reduced in possible capture of the prey. It is interesting to note that dazzle camouflage does not seem to be used. More studies need to be conducted looking at motion camouflage in cuttlefish to determine whether or not these theories hold true in a more complex environment.

The experiments outlined in this thesis have investigated a range of different environmental factors and their influence on camouflage. It has highlighted the importance of not only thinking of the camouflage produced, but also the visual input and the environmental pressures that drive camouflage decisions in cuttlefish. In addition, it has raised new questions to be investigated and ideas to be discussed.

Principal component	PC1		PC2		PC3	
White Square	Proximity	Stone	Proximity	Stone	Proximity	Stone
	LL/Turbid	Projector	LL/Turbid	Projector	LL/Turbid	Projector
White Head Bar	Proximity	Stone	Proximity	Stone	Proximity	Stone
	LL/Turbid	Projector	LL/Turbid	Projector	LL/Turbid	Projector
White Land mark spots (white square			Proximity	Stone	Proximity	Stone
			LL/Turbid	Projector	LL/Turbid	Projector
White Splotches			Proximity	Stone	Proximity	Stone
			LL/Turbid	Projector	LL/Turbid	Projector
Mantle Margin Scalloping	Proximity	Stone	Proximity	Stone	Proximity	Stone
	LL/Turbid	Projector	LL/Turbid	Projector	LL/Turbid	Projector
Paired mantle spots (posterior)	Proximity	Stone	Proximity	Stone	Proximity	Stone
	LL/Turbid	Projector	LL/Turbid	Projector	LL/Turbid	Projector
Paired mantle spots (anterior)	Proximity	Stone	Proximity	Stone		
	LL/Turbid	Projector	LL/Turbid	Projector		
White Mantle Bar	Proximity	Stone			Proximity	Stone
	LL/Turbid	Projector			LL/Turbid	Projector
Anterior head bar	Proximity	Stone	Proximity	Stone		
	LL/Turbid	Projector	LL/Turbid	Projector		
Anterior transverse Mantle bar	Proximity	Stone	Proximity	Stone	Proximity	Stone
	LL/Turbid	Projector	LL/Turbid	Projector	LL/Turbid	Projector
Posterior head bar	Proximity	Stone				
	LL/Turbid	Projector				
Cuttlebone	Proximity	Stone	Proximity	Stone	Proximity	Stone
	LL/Turbid	Projector	LL/Turbid	Projector	Stone	Projector
Posterior transverse mantle bar	Proximity	Stone			Proximity	Stone
	LL/Turbid	Projector			LL/Turbid	Projector
Median Mantle Stripe	Proximity	Stone	Proximity	Stone	Proximity	Stone
	LL/Turbid	Projector	LL/Turbid	Projector	LL/Turbid	Projector
White Posterior triangle	Proximity	Stone	Proximity	Stone		
	LL/Turbid	Projector	LL/Turbid	Projector		
Dark arms	Proximity	Stone	Proximity	Stone	Proximity	Stone
	LL/Turbid	Projector	LL/Turbid	Projector	LL/Turbid	Projector
Anterior Mantle Bar	Proximity	Stone				
	LL/Turbid	Projector				
Posterior Mantle Bar	Proximity	Stone				
	LL/Turbid	Projector				
Mottle	Proximity	Stone				
	LL/Turbid	Projector				
White landmark spots (head)	Proximity	Stone	Proximity	Stone	Proximity	Stone
	LL/Turbid	Projector	LL/Turbid	Projector	LL/Turbid	Projector
White Landmark spots (arms)			Proximity	Stone		
			LL/Turbid	Projector		
White major lateral papillae			Proximity	Stone	Proximity	Stone
			LL/Turbid	Projector	LL/Turbid	Projector

-1	-0.75	-0.5	-0.25	0	0.25	0.5	0.75	1

Figure 7.1: Principal Component matrix comparison of stationary chapter component outputs

8.References

Abrahams, M. V. and Kattenfeld, M. G. (1997). The role of turbidity as a constraint on predator-prey interactions in aquatic environments. *Behavioral Ecology and Sociobiology*, *40*(3), 169-174.

Adamo, S. A., Ehgoetz, K., Sangster, C. and Whitehorne, I. (2006). Signaling to the enemy? Body pattern expression and its response to external cues during hunting in the cuttlefish Sepia officinalis (Cephalopoda). *The Biological Bulletin*, *210*(3), 192-200.

Allen, J. J., Mäthger, L. M., Buresch, K. C., Fetchko, T., Gardner, M. and Hanlon, R. T. (2010). Night vision by cuttlefish enables changeable camouflage. *Journal of Experimental Biology*, *213*(23), 3953-3960.

Barbosa, A., Allen, J.J., Mäthger, L.M. and Hanlon, R.T. (2012), January. Cuttlefish use visual cues to determine arm postures for camouflage. In *Proc. R. Soc. B 279* (1726), 84-90.

Barbosa, A., Litman, L. and Hanlon, R. T. (2008). Changeable cuttlefish camouflage is influenced by horizontal and vertical aspects of the visual background. *Journal of Comparative Physiology A*, *194*(4), 405-413.

Barbosa, A., Mäthger, L. M., Buresch, K. C., Kelly, J., Chubb, C., Chiao, C. C. and Hanlon, R. T. (2008). Cuttlefish camouflage: the effects of substrate contrast and size in evoking uniform, mottle or disruptive body patterns. *Vision research*, *48*(10), 1242-1253.

Boal, J.G., Wittenberg, K.M. and Hanlon, R.T. (2000). Observational learning does not explain improvement in predation tactics by cuttlefish (Mollusca: Cephalopoda). *Behavioural processes*, *52*(2), pp.141-153

Buresch, K.C., Mäthger, L.M., Allen, J.J., Bennice, C., Smith, N., Schram, J., Chiao, C.C., Chubb, C. and Hanlon, R.T. (2011). The use of background matching vs. masquerade for camouflage in cuttlefish Sepia officinalis. *Vision research*, *51*(23),.2362-2368.

Buresch, K. C., Ulmer, K. M., Akkaynak, D., Allen, J. J., Mäthger, L. M., Nakamura, M. and Hanlon, R. T. (2015). Cuttlefish adjust body pattern intensity with respect to substrate intensity to aid camouflage, but do not camouflage in extremely low light. *Journal of Experimental Marine Biology and Ecology*, *462*, 121-126.

Caro, T. (2005). *Antipredator defenses in birds and mammals*. University of Chicago Press.

Cartron, L., Josef, N., Lerner, A., McCusker, S. D., Darmaillacq, A. S., Dickel, L. and Shashar, N. (2013). Polarization vision can improve object detection in turbid waters by cuttlefish. *Journal of Experimental Marine Biology and Ecology*, *447*, 80-85.

Compagno, L. (2001) "FAO Species catalogue for fisheries purposes.". *Food and Agriculture Organization of the United Nations, Rome* No4 Vol 1.

Chiao, C. C., Chubb, C. and Hanlon, R. T. (2007). Interactive effects of size, contrast, intensity and configuration of background objects in evoking disruptive camouflage in cuttlefish. *Vision research*, *47*(16), 2223-2235.

Chiao, C. C., Chubb, C., Buresch, K., Siemann, L. and Hanlon, R. T. (2009). The scaling effects of substrate texture on camouflage patterning in cuttlefish. *Vision research*, *49*(13), 1647-1656.

Chiao, C. C., Chubb, C., Buresch, K. C., Barbosa, A., Allen, J. J., Mäthger, L. M. and Hanlon, R. T. (2010). Mottle camouflage patterns in cuttlefish: quantitative characterization and visual background stimuli that evoke them. *Journal of Experimental Biology*, *213*(2), 187-199.

Cooke, Gavan M., and Belinda M. Tonkins. (2015) Behavioural indicators of welfare exhibited by the common European. *Journal of Zoo and Aquarium Research* 3.4: 157.

Confer, J. L. and Blades, P. I. (1975). Omnivorous zooplankton and planktivorous fish. *Limnology and Oceanography*, *20*(4), 571-579.

Cott, H.B.(1940). *Adaptive coloration in animals*. London.

Edmunds, M. (1974). *Defence in animals: a survey of anti-predator defences*. Longman Publishing Group.

Endler, John A. (1978) "A predator's view of animal color patterns." *Evolutionary biology*. Springer US,319-364.

Endler, J. A. (1984). Progressive background in moths, and a quantitative measure of crypsis. *Biological Journal of the Linnean Society*, *22*(3), 187-231.

Endler, J. A. (1990). On the measurement and classification of colour in studies of animal colour patterns. *Biological Journal of the Linnean Society*,*41*(4), 315-352.

Endler, J. A. (1991). Interactions between predators and prey. *Behavioural ecology: an evolutionary approach*, *3*, 169-196.

Ferguson, G. P and Messenger, J. B. (1991). A countershading reflex in cephalopods. *Proceedings of the Royal Society of London B: Biological Sciences*, *243*(1306), 63-67.

Fiorito, G., Affuso, A., Basil, J., Cole, A., de Girolamo, P., D'Angelo, L., Dickel, L., Gestal, C., Grasso, F., Kuba, M. and Mark, F. (2015). Guidelines for the Care and Welfare of Cephalopods in Research–A consensus based on an initiative by CephRes, FELASA and the Boyd Group. *Laboratory animals*, *49*(2_suppl), 1-90.

Gardner, M.B. (1981). Effects of turbidity on feeding rates and selectivity of bluegills. *Transactions of the American Fisheries Society*, *110*(3),446-450.

Hacker, S.D. and Madin, L.P.(1991). Why habitat architecture and color are important to shrimps living in pelagic Sargassum: use of camouflage and plant-part mimicry. *Marine ecology progress series. Oldendorf*, *70*(2),143-155.

Hailman, J. P. (1977). *Optical signals: animal communication and light*. Indiana U Press.

Hanlon, R. (2007). Cephalopod dynamic camouflage. *Current Biology*, *17*(11), R400-R404.

Hanlon, R. T. and Messenger, J. B. (1988). Adaptive coloration in young cuttlefish (Sepia officinalis L.): the morphology and development of body patterns and their relation to behaviour. *Philosophical Transactions of the Royal Society of London B: Biological Sciences*, *320*(1200), 437-487.

Hanlon, R. T. and Messenger J.B (1996). *Cephalopod behaviour*. Cambridge University Press,

Hochberg, F. G. and O. Kinne (1990). "Diseases of Mollusca: Cephalopoda. Diseases caused by protistans and metazoans." *Diseases of marine animals Volume III: Introduction, Cephalopoda, Annelida, Crustacea, Chaetognatha, Echinodermata, Urochordata.*

Home office (2014), Code of practice for the housing and care of animals bred, supplied or used for scientific purposes

Iglesias, José, Lidia Fuentes, and Roger Villanueva, eds. (2014) *Cephalopod culture*. Springer Science and Business Media,

Johnsen, Sönke (2012). *The optics of life: a biologist's guide to light in nature*. Princeton University Press,

Josef, N., Amodio, P., Fiorito, G. and Shashar, N.(2012). Camouflaging in a complex environment—octopuses use specific features of their surroundings for background matching. *PloS one*, *7*(5), p.e37579.

Josef, N., Berenshtein, I., Fiorito, G., Sykes, A.V. and Shashar, N. (2015). Camouflage during movement in the European cuttlefish (Sepia officinalis). *Journal of Experimental Biology*, *218*(21), 3391-3398.

Josef, N., Berenshtein, I., Rousseau, M., Scata, G., Fiorito, G. and Shashar, N. (2016). Size Matters: Observed and Modeled Camouflage Response of European Cuttlefish (Sepia officinalis) to Different Substrate Patch Sizes during Movement. *Frontiers in Physiology*, *7*.

Josef, N., Mann, O., Sykes, A. V., Fiorito, G., Reis, J., Maccusker, S. and Shashar, N. (2014). Depth perception: cuttlefish (Sepia officinalis) respond to visual texture density gradients. *Animal cognition*, *17*(6), 1393-1400.

Kelman E (2007) Visual perception and camouflage of the common cuttlefish sepia officinalis. Doctoral thesis. University of Sussex

Kelman, E. J., Osorio, D. and Baddeley, R. J. (2008). A review of cuttlefish camouflage and object recognition and evidence for depth perception.*Journal of Experimental Biology*, *211*(11), 1757-1763.

Land, Michael F. and Dan-Eric Nilsson. (2012) *Animal eyes*. Oxford University Press,

Langridge, K. V. (2006). Symmetrical crypsis and asymmetrical signalling in the cuttlefish Sepia officinalis. *Proceedings of the Royal Society of London B: Biological Sciences*, *273*(1589), 959-967.

Langridge, K. V., Broom, M. and Osorio, D. (2007). Selective signalling by cuttlefish to predators. *Current Biology*, *17*(24), R1044-R1045.

Langridge, K. V. (2009). Cuttlefish use startle displays, but not against large predators. *Animal Behaviour*, *77*(4), 847-856.

Lythgoe, J. N. (1979). *Ecology of vision*. Clarendon Press; Oxford University Press.

Marshall, N. J., Jennings, K., McFarland, W. N., Loew, E. R. and Losey, G. S. (2003). Visual biology of Hawaiian coral reef fishes. III. Environmental light and an integrated approach to the ecology of reef fish vision. *Copeia*,*2003*(3), 467-480.

Meager, J. J. and Batty, R. S. (2007). Effects of turbidity on the spontaneous and prey-searching activity of juvenile Atlantic cod (Gadus morhua).*Philosophical Transactions of the Royal Society of London B: Biological Sciences*, *362*(1487), 2123-2130.

Merilaita, S. and Tullberg, B. S. (2005). Constrained camouflage facilitates the evolution of conspicuous warning coloration. *Evolution*, *59*(1), 38-45.

Navarro, Juan Carlos, Óscar Monroig and António V. Sykes (2014). "Nutrition as a key factor for cephalopod aquaculture." *Cephalopod Culture*. Springer Netherlands,. 77-95.

Messenger, J. B. (1968). The visual attack of the cuttlefish, Sepia officinalis.*Animal Behaviour*, *16*(2), 342-357.

Messenger, J. B. (2001). Cephalopod chromatophores: neurobiology and natural history. *Biological Reviews*, *76*(4), 473-528.

Nalbach, H.O., Nalbach, G. and Forzin, L. (1989). Visual control of eye-stalk orientation in crabs: vertical optokinetics, visual fixation of the horizon, and eye design. *Journal of comparative Physiology A*, *165*(5),577-587.

Norris, K. S. and Lowe, C. H. (1964). An Analysis of Background Color-Matching in Amphibians and Reptiles. *Ecology*, *45*(3), 565-580.

Oestmann, D.J., Scimeca, J.M., Forsythe, J., Hanlon, R. and Lee, P. (1997). Special considerations for keeping cephalopods in laboratory facilities. *Journal of the American Association for Laboratory Animal Science*, *36*(2), pp.89-93.

Oliva, D., Medan, V. and Tomsic, D. (2007). Escape behavior and neuronal responses to looming stimuli in the crab Chasmagnathus granulatus (Decapoda: Grapsidae). *Journal of Experimental Biology*, *210*(5), 865-880.

Pignatelli, V., Temple, S. E., Chiou, T. H., Roberts, N. W., Collin, S. P. and Marshall, N. J. (2011). Behavioural relevance of polarization sensitivity as a target detection mechanism in cephalopods and fishes. *Philosophical Transactions of the Royal Society of London B: Biological Sciences*,*366*(1565), 734-741.

Ranåker, L., Jönsson, M., Nilsson, P.A. and Brönmark, C. (2012). Effects of brown and turbid water on piscivore–prey fish interactions along a visibility gradient. *Freshwater Biology*, *57*(9), 1761-1768.

Rosenthal, G. G. (2007). Spatiotemporal dimensions of visual signals in animal communication. *Annual Review of Ecology, Evolution, and Systematics*, 155-178.

Ruxton, G. D., Sherratt, T. N. and Speed, M. P. (2004). *Avoiding attack: the evolutionary ecology of crypsis, warning signals and mimicry*. Oxford University Press.

Schechner, Y. Y., Narasimhan, S. G. and Nayar, S. K. (2003). Polarization-based vision through haze. Appl. Optics 42, 511-525.

Shohet, A. J., Baddeley, R. J., Anderson, J. C., Kelman, E. J. and Osorio, D. (2006). Cuttlefish responses to visual orientation of substrates, water flow and a model of motion camouflage. *Journal of Experimental Biology*,*209*(23), 4717-4723.

Skelhorn, J., Rowland, H.M., Delf, J., Speed, M.P. and Ruxton, G.D(2011). Density-dependent predation influences the evolution and behavior of masquerading prey. *Proceedings of the National Academy of Sciences*, *108*(16), pp.6532-6536.

Slater M., Tyson P., Tuson K. and Rundle J. (2013) Management Guidelines for the Welfare of Zoo and Aquarium Animals – Cuttlefish (Sepia officinalis). London: BIAZA

Srinivasan, M. V. and Davey, M. (1995). Strategies for active camouflage of motion. *Proceedings of the Royal Society of London B: Biological Sciences*,*259*(1354), 19-25.

Stevens, Martin. (2007): Predator perception and the interrelation between different forms of protective coloration. *Proceedings of the Royal Society of London B: Biological Sciences* 274.1617 1457-1464.

Stevens, Martin and Sami Merilaita, eds.(2011). *Animal camouflage: mechanisms and function*. Cambridge University Press,

Stevens, M., Searle, W. T. L., Seymour, J. E., Marshall, K. L. and Ruxton, G. D. (2011). Motion dazzle and camouflage as distinct anti-predator defenses.*BMC biology*, *9*(1), 1.

Stevens, M., Stubbins, C. L. and Hardman, C. J. (2008). The anti-predator function of 'eyespots' on camouflaged and conspicuous prey. *Behavioral Ecology and Sociobiology*, *62*(11), 1787-1793.

Stramski, Dariusz and Dale A. Kiefer (1991). Light scattering by microorganisms in the open ocean. *Progress in Oceanography* 28.4 : 343-383.

Stuart-Fox, D., Moussalli, A. and Whiting, M.J. (2008). Predator-specific camouflage in chameleons. *Biology Letters*, *4*(4), 326-329.

Sykes, António V. and Camino Gestal (2014). Welfare and Diseases Under Culture Conditions. *Cephalopod Culture*. Springer Netherlands,. 97-112.

Ulmer, K. M., Buresch, K. C., Kossodo, M. M., Mäthger, L. M., Siemann, L. A. and Hanlon, R. T. (2013). Vertical visual features have a strong influence on cuttlefish camouflage. *The Biological Bulletin*, *224*(2), 110-118.

Utne, A.C.W. (1997). The effect of turbidity and illumination on the reaction distance and search time of the marine planktivore Gobiusculus flavescens. *Journal of Fish biology*, *50*(5), 926-938.

Utne-Palm, A. C. (2002). Visual feeding of fish in a turbid environment: physical and behavioural aspects. *Marine and Freshwater Behaviour and Physiology*, *35*(1-2), 111-128.

Zylinski S (2009) Cuttlefish: Camouflage and visual imaging in the marine environment. Doctoral thesis University of Sussex

Zylinski, S., Osorio, D. and Shohet, A. J. (2009). Perception of edges and visual texture in the camouflage of the common cuttlefish, Sepia officinalis. *Philosophical Transactions of the Royal Society of London B: Biological Sciences*, *364*(1516), 439-448.

Zylinski, S., Osorio, D. and Shohet, A.J. (2009b). Cuttlefish camouflage: context-dependent body pattern use during motion. *Proceedings of the Royal Society of London B: Biological Sciences*, *276*(1675), .3963-3969.